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Resumen por el autor, Peter Okkelberg,  
Universidad de Michigan.

Historia de las células germinales de la lamprea de arroyo,  
*Entosphenus wilderi* (Gage) hasta el periodo de  
diferenciación.

Las células germinativas se segregan de las restantes en una época muy temprana de la vida del animal, aun antes de haberse formado definitivamente las hojas germinales. Pueden reconocerse por primera vez cuando el mesodermo se separa del endodermo (embrión de unas 191 horas de edad). Las células masculinas y femeninas definitivas no reconocen otro origen que el de las células germinales primordiales; las células germinales tampoco toman parte en la formación de estructuras somáticas. Muchas de las células germinales degeneran y desaparecen en cada individuo.

Durante el periodo de diferenciación sexual las células germinales de cada glándula germinal son claramente de dos tipos: Unas que presentan marcada tendencia hacia una división continua (células catabólicas) y otras que tienden a crecer (células anabólicas). El autor considera a las primeras como poseedoras de una potencialidad masculina, y a las segundas como femeninas. La proporción relativa de células anabólicas y catabólicas determina si la larva ha de ser un macho o una hembra. Las observaciones del autor parecen justificar la conclusión de que cada larva de esta especie lleva la potencialidad para producir los dos sexos, y que el sexo, por consiguiente, no se fija de modo irrevocable en el momento de la fecundación. En el trabajo se describen las diversas estructuras nucleares y citoplásmicas y los cambios que sufren durante las diversas fases del desarrollo.

Translation by José F. Nonidez  
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# THE EARLY HISTORY OF THE GERM CELLS IN THE BROOK LAMPREY, *ENTOSPHEUS WILDERI* (GAGE), UP TO AND INCLUDING THE PERIOD OF SEX DIFFERENTIATION

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FOUR TEXT FIGURES AND TWELVE PLATES. (SEVENTY-EIGHT FIGURES)

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## INTRODUCTION

Owing to their bearing on current theoretical questions, certain phases of the germ-cell cycle in vertebrates have received more attention than others. Maturation has been fully studied because it appears to involve a redistribution of parental hereditary factors. The small size and the brief maturation period of the spermatocytes make it possible to obtain all stages very easily on a single slide, whereas much time and material are needed to obtain the stages of maturation in the much larger oocytes. Since it usually is assumed that the maturation phenomena are practically identical in the two sexes, the male has been commonly selected for the study of this period because it presents lesser technical difficulties. A second phase on which attention has been focused in recent years, because of its bearing on the question of germ-plasm continuity, is that of the origin and early development of the germ cells. The period of differentiation of the male and female sex cells from the primordial germ cells has been relatively neglected, apparently because of the belief that in all animals sex is irrevocably determined at or before fertilization through the agency of sex chromosomes. The result is that we have much literature on isolated periods in the germ-cell history of vertebrates, chiefly in the male sex, but few comprehensive accounts.

The work of Brock ('81), Schreiner ('04), R. Hertwig ('05, '06, '07), and others has shown that in both sexes of certain species of vertebrates there is a tendency toward hermaphroditism at the period of differentiation of the primordial germ cells—an indication that sex is not irrevocably determined at the time of fertilization. This fact and the lack of a complete account of the germ-cell cycle in any vertebrate led me to undertake a study of the whole history of the germ cells in both sexes in the American brook lamprey, *Entosphenus wilderi* (Gage), in which species there is a decided tendency toward a condition of juvenile hermaphroditism. This seemed the more worth while because the lampreys and the hag-fishes are now elevated to a separate vertebrate class, and in this class very little work has been done on the germ-cell cycle.

I wish to express my sincere appreciation of the help and encouragement received from Prof. Jacob Reighard during the progress of the work.

#### SPAWNING HABITS AND LIFE-CYCLE OF ENTOSPHEUS WILDERI

##### *A. Spawning habits*

*Entosphenus wilderi* is abundant about Ann Arbor in several streams tributary to the Huron River. All the material used in the present study was collected from Honey Creek, a small stream about four miles west of the city.

On the average, the first lampreys appear on the spawning grounds at Ann Arbor about the 10th of April. Dean and Sumner ('97) report them spawning on the 16th of April in New York City. According to Gage ('93), they spawn between the 8th and 20th of May at Cayuga Lake, New York. The time of spawning is undoubtedly dependent upon temperature as determined by the progress of the season. The temperature of the water in Honey Creek, when the lampreys first appear in the spring, ranges from 13°C. to 14½°C. The water is warmer down stream than farther up, and it is usually down stream that the lampreys are first observed. This, of course, may be due to the

fact that the temperature is higher in this part of the stream, but it may also be correlated with the fact that many larvae have been carried down stream during successive years of their life, so that the number ready to transform into adults is greater in the lower part of the stream. Evidence for this is the fact that older larvae are usually obtained from the lower part of the stream, while it is very seldom that any large or full-grown larvae are found in the upper part.

I have found that the males appear on the spawning grounds before the females. Usually also only males are found in the nests early in the morning and late at night. That the males appear earlier in the season than the females was observed also by Young and Cole ('00), and, according to Surface ('97), the same is true of the lake lamprey. It is therefore necessary to collect the animals when they are spawning under optimum conditions in order to obtain reliable data concerning sex ratio. Dean and Sumner ('97) report more males than females in the proportion of five to one. It is easy to see how one might get such results from collections early in the season or at certain times of the day. Loman ('12) found no such disproportion of sexes in the European brook lamprey and neither have I found in the American brook lamprey an excess of one sex over the other when spawning conditions were at their optimum.

It is claimed by Loman ('12) that in the European brook lamprey internal fertilization takes place. The same idea has been advanced by Ferry ('83) in regard to the marine lamprey. To test whether or not spawning females of *Entosphenus wilderi* contained spermatozoa, the urogenital sinus, as well as the posterior portion of the body cavity, of a large number of living specimens was examined. In no case could any spermatozoa be found, nor did the eggs from the posterior part of the body cavity develop without the addition of sperm after being stripped. This shows clearly that, if internal fertilization occurs in this species, it must be only as a rare exception. The spermatozoa of the brook lamprey are motile for less than a minute after being shed into the water, but they are extremely active and are extruded simultaneously with the eggs. It is, therefore, inherently prob-



able that fertilization of nearly all the eggs is insured. In fact, all eggs collected from the stream after deposition are found to be developing.

### *B. Life-cycle*

The life-cycle of the lamprey may be divided into three main periods: the embryonic, the larval, and the adult (table 2). There is no sharp structural change between the first and second periods, but the first period may arbitrarily be considered as ending at the time of hatching and the larval period at the time of metamorphosis. The duration of the embryonic period depends on the temperature of the water during development. Balfour ('81) estimated the length of the period to be from thirteen to twenty-one days in *Petromyzon planeri*. A. Müller ('56) says that in the European brook lamprey (*kleines Neunauge*) hatching takes place about eighteen days after fertilization. I have found that when the temperature of the water ranges from 17 to 20°C., the artificially fertilized eggs of *Entosphenus wilderi* hatch in the laboratory in about ten to twelve days. There may, however, be a great deal of variation in the rate of development, even in the same brood of eggs when kept in the same dish.

The length of the larval period is not known from direct observation. It would be difficult in the laboratory, if not impossible, to raise the larvae to the time of metamorphosis, and if one were successful in doing so, he could not be certain that the larval period was of the same length as in the natural habitat. Various estimates have been given. A. Müller ('56) states that metamorphosis probably does not take place before the fourth year in the case of the European brook lamprey. Loman ('12) found larvae of four sizes, which he took to represent four successive generations, or four years of development before metamorphosis. Lubosch ('03) estimates the adult age to be four years, the estimate being based on the size of the larvae found. According to him, they attain an average size of 5 cm. during the first year; 10, during the second year, and 15 to 18 cm. during the third year, or just before metamorphosis.

Schaffner ('02) makes the following statement concerning the age of *Entosphenus wilderi* at sexual maturity: "The larvae taken a few days preceding the breeding season can be grouped under three divisions as regards size, and we took this to indicate that it required three years for the complete development of the larvae." The sizes ranged as follows: Those almost fully developed, 17 to 20 cm.; a second group, 9 to 11 cm.; and a third group, 3 to 6 cm.

The variation in size of the larvae of each year (figs. 1 to 4) is so great that from any one catch a continuous series may be selected. The measurements of seventy-seven adult males taken at random show a range in total length from 13.5 cm. to 19 cm., and an average length of 16.12 cm. Similar measurements of sixty-five females give a range from 13.3 cm. to 18.5 cm., with an average length of 15.61 cm. It thus appears that size is so variable in the adults and larvae of this species that it cannot be regarded as a reliable index of the age of the individual.

I have sought, by means of average curves, to determine the length of the life-cycle in *Entosphenus wilderi*. Such curves were obtained from measurements of the lengths of larvae collected during single months of the year. When the number of specimens was small, the sizes graded into each other so that no suggestive curves were obtained. The greatest number of specimens was collected in the month of August, and the curve represented in figure 1 is the result of measurements of 167 larvae from this period. The first hump of the curve, with its peak at 2 cm., represents larvae hatched in the preceding April, and, therefore, about four months old. Most of these larvae are about 2 cm. long. The second hump has its peak at 5 cm., and presumably represents larvae which are about one year and four months old. The third, fourth, and fifth humps with their peaks at 8, 12, and 18 cm. presumably represent larvae  $2\frac{1}{3}$ ,  $3\frac{1}{3}$ , and  $4\frac{1}{3}$  years old, respectively. The larvae of the latter age would undoubtedly transform into adults during the fall, as they have already attained their full larval size.

Another series of larvae from the month of February was measured and the results are represented in the curve shown in

figure 2. The number of specimens measured in this case is 130. It will be seen that this curve has four distinct humps. The larvae, which in the first curve were represented by the fifth hump, have supposedly metamorphosed and are at this time of the year in the adult form. The other humps of the curve represent larvae about one, two, three, and four years old.

Two other curves are shown, each of which is constructed from combined collections of three successive months. The curve for April, May, and June (fig. 3) has the peaks of the humps at 3, 9, 12, and about 14 cm., respectively. The humps represent larvae one, two, three, and four years old. At this time of the year adults are found and also larvae that have just hatched, but these are not included in the graphs. The other curve from the months of July, August, and September shows practically the same thing. It appears from these curves that *Entosphenus wilderi* probably attains an age of five years before it is sexually mature. This does not exclude the possibility that individuals may reach their full size in four years.

The rate of growth varies greatly, as shown in figures 1 to 4, where individuals grouped in each hump of the curves are presumably of the same age. The twenty-six larvae represented by the first hump in figure 1 range in length from 15 mm. to 32.5 mm. and have an average length of about 24 mm. As shown by the curves, the same variation in the rate of growth exists among older larvae. This probably is due largely to differences in the food supply in different parts of the stream and to the inactivity of the larvae, which keeps them from seeking out the optimum environmental conditions. It was found, for instance, when the larvae were kept under observation in the laboratory, that they remained in their burrows for days at a time, even though they were kept in a small dish without running water. It was only when the water became warm and stale that they came out. In the stream the water is cool and well aerated, so that it is doubtful if the larvae ever come out unless disturbed by torrents after heavy rains and during spring thaws.

The time of metamorphosis probably varies somewhat in different species and also in different localities. Balfour ('81) says

that it takes place from August to January in *Petromyzon planeri*. Gage ('93) believes it occurs about from August to September or October in *Petromyzon branchialis*, and estimates the time required for metamorphosis as probab'y ten to twenty-six days. Reese ('00) found that larvae of the same species, which were kept in the laboratory, metamorphosed about the 20th of October. Schneider ('79) found metamorphosing larvae of *Petromyzon planeri* as early as the middle of August. I have found larvae of *Entosphenus wilderi* metamorphosing in the brook during the months of August and September, but only four specimens have been obtained; two on August 16th, one on August 23d, and one on September 13th. No direct observations have been made on the length of time required for metamorphosis, but since adults have not been found during May, June, and July, the three months following the spawning season; since metamorphosing individuals occur in late August and early September while adults are obtained during the other months of the year, it is likely that the usual time for metamorphosis in this species is in August and September, and that it requires only a short time.

*Entosphenus wilderi* reaches its full length in the larval state. After metamorphosis the germ glands grow very rapidly and soon fill up the whole body cavity. The intestine atrophies and becomes so small that it can be distinguished only with difficulty in cross-sections of the body. After transformation no food is taken by the animal. The whole metabolism of the body seems to be changed and all the resources possessed by the individual are used toward maturing the sexual products. After the spawning season the adults die within a very short time. Dead lampreys may sometimes be found along the stream, and crayfish have been found feeding on the dead bodies lying on the bottom of the stream.

It is remarkable that during the period of its adult life, extending from August or September to the following April, the lamprey takes no food. During this time the germ gland increases greatly in size and most of the material for its growth must be furnished by the fat body (*corpus adiposum*) and by the other

tissues of the body. Metamorphosis seems to be the beginning of the death process, since at this time the intestine and the portal vein degenerate, thus making it impossible for the animal to feed again. The metabolic processes taking place in the adult must be concerned largely with material already present in the body at the time of metamorphosis. It is not unlikely, however, that water and some nutritive substances may be absorbed during adult life through the external surface of the body. Pütter ('09) found that sea-water and probably also fresh water contains amino-acids, oils, and carbohydrates, and that many aquatic animals absorb nutrition from solution, thus rendering them only in part dependent upon plankton. Alcock ('99) found that the larvae of the lamprey secrete an enzyme through the skin which has some digestive action on bacteria that might attack them in their burrows. This renders it probable that some food may be absorbed through the skin of the larvae and perhaps of the adult.

#### MATERIALS AND METHODS

Adults have been obtained at all seasons of the year except during the three months following the spawning period, but only those taken during the spawning season contained fully matured sex cells. In the laboratory the adults must be kept in running water, but the larvae live for months in standing tap-water if it be kept cool. Early larval stages are best obtained from artificially fertilized eggs. The largest percentage of fertilization is had from eggs of females taken at the height of the spawning season and used at once. The fertilized eggs are placed in tap-water in covered bacteria dishes and require no more care than an occasional change of water, which should be made without considerable change of temperature. Larvae from such eggs have been kept without much difficulty in the same dishes for as long as forty days, i.e., until most of the yolk is absorbed. Older larvae were obtained from the brook at all seasons and for a period of about four years.

By recording the moment of fertilization and by watching the progress of development, it was possible to obtain any

desired stage. The older larvae collected from the stream were usually anaesthetized and put into the fixing solution as soon as they reached the laboratory. Precaution was taken to insure a rapid fixation of the germ gland.

Various fixing reagents were used, but the best results were obtained with Flemming's, Meves', and Bouin's solutions. For embryos in which there was a great deal of yolk, Bouin's solution gave very satisfactory results; it also gave uniformly good results for all other stages. For certain nuclear and cytoplasmic structures, Flemming's and Meves' solutions were more satisfactory. After fixation the material was left in alcohol for a few days and then imbedded. The results were not as good if the material had remained in alcohol for a long time.

Haemalum with a counterstain of orange G gave the best results for early stages in which a great deal of yolk was present. This combination gave the yolk a yellowish or brownish tint, while the cytoplasm was stained more or less bluish. For later stages iron haematoxylin was used almost exclusively, either alone or with a counterstain of eosin or Licht-grün.

#### HISTORY OF THE GERM CELLS

##### *A. General outline of the whole germ-cell history in animals; special outline for *Entosphenus wilderi**

In table 1 an outline is given of the different periods, as defined by various writers, in the development of the germ cells of vertebrates. The scheme recognizes, in the column headed 'periods in the germ-cell cycle,' an early segregation of the germ cells and the development of all the definitive germ cells from the early segregated cells. The table also admits the possibility of two alternatives as to the origin of sex in the young animal. There may be distinct male and female individuals from the beginning of embryonic development, as shown in columns I and II of the table, in which case sex is dependent on an hereditary factor or on other factors that influence the germ cells at or before fertilization. On the other hand, the young animal may be indifferent as to sex, as shown in column III

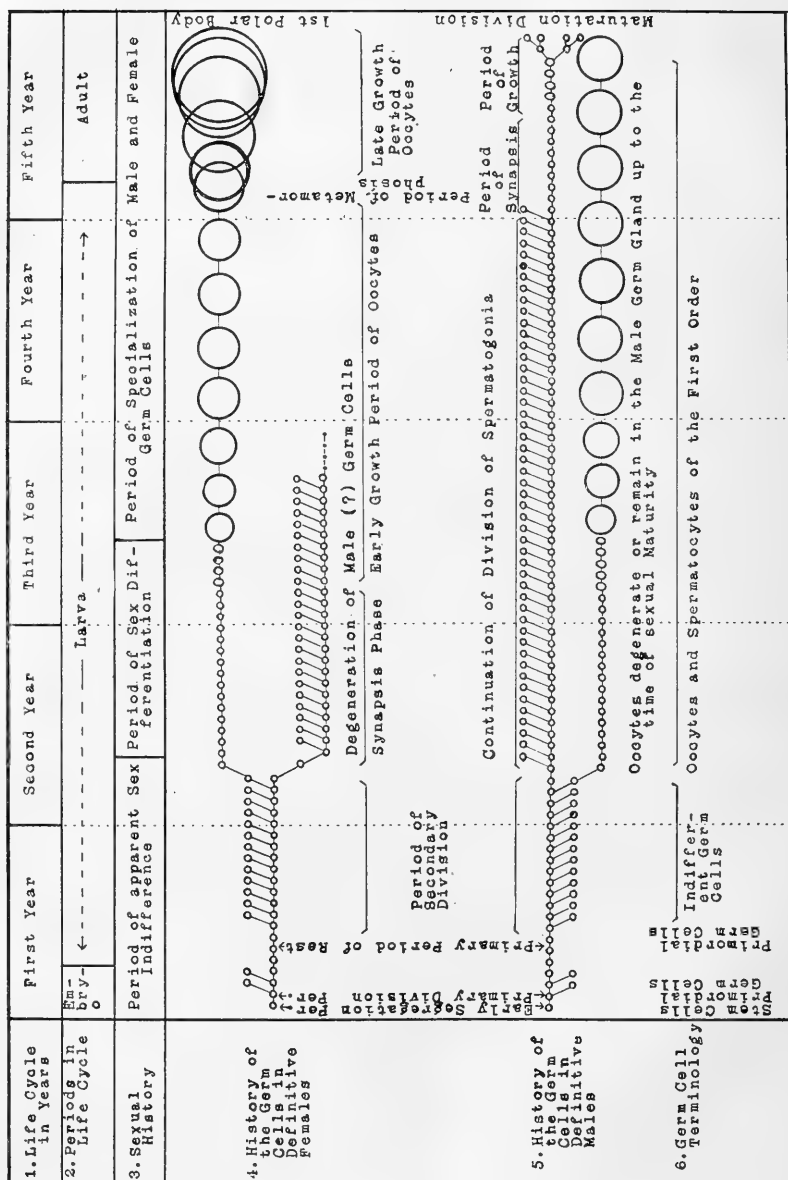


TABLE 1

*A scheme representing in outline the different periods in the development of the germ cells in vertebrates, and the terminology that might be employed when the sexes are distinct from the beginning of development (columns I and II) and when the young animal appears indifferent as to sex (column III). See explanation in text*

PERIODS IN THE GERM-CELL CYCLE	GERM-CELL TERMINOLOGY			
	Sex determined at or before fertilization		Sex determined after fertilization	
	I	II	III	
	Embryo and larva male	Embryo and larva female	Embryo and larva indifferent	
1. Period of early segregation	Primary spermatogonia	Primary oogonia	Stem cell	
2. Period of primary division	Primary spermatogonia	Primary oogonia	Primordial germ cells	
3. First period of rest	Primary spermatogonia	Primary oogonia	Indifferent germ cells	
4. Period of secondary division	Secondary spermatogonia	Secondary oogonia	Indifferent germ-cells	
5. Period of maturation and growth	Primary spermatocytes	Primary oocytes	Male	Female
			Primary spermatocytes	Primary oocytes
	Spermatids	Ova	Spermatids	Ova
6. Period of cell metamorphosis			Spermatozoa	

TABLE 2  
Outline of the history of the germ cells of the brook lamprey in relation to the life cycle of the animal. The germ cells are represented by circles



of the table, in which case factors influencing the animal during development may be responsible for the resulting sex. The possibility is not excluded of sex being the result of the joint action of hereditary and external factors.

Whether the germ cells of the larval lamprey eventually give rise to ova or to spermatozoa, their early history appears to be the same. Sooner or later some of the primordial germ cells transform into oocytes in practically all the larvae, irrespective of whether the larvae which bear them eventually become males or females. In the present work the history of the germ cells has been studied up to a period when males and females can be distinguished by an examination of the germ glands.

The scheme in table 2 presents in a graphic form the history of the germ cells in the lamprey in both males and females in relation to the development of the body. As here shown, the life of the animal extends over a period of five years, and only a small part of the life-cycle is spent in the adult stage. The scheme also forms a basis for the terminology employed in the subsequent pages.

*B. Origin and early history of the germ cells up to the beginning of sex differentiation*

1. *Observations on Entosphenus wilderi.* During cleavage and gastrulation in the lamprey all cells are more or less laden with yolk and, if the germ cells are segregated at this time, no character has been discovered by means of which they may be distinguished from other cells. But after the mesoderm begins to separate from the entoderm certain large cells occur which may be identified as the primordial germ cells.

a. Embryo 191 hours old (fig. 5). A camera-lucida drawing of an embryo 191 hours old (two or three days before hatching) is shown in figure 5, and figure 11 shows a section along the line *ab* of figure 5. In this embryo the mesoderm has already separated from the entoderm cranially, but at the caudal end, i.e., in the region from which the section (fig. 11) was taken, there seems as yet to be no division line between the two layers. The mesentoderm in this region extends dorsad as two ridges, one

on either side of the nerve cord and notochord, and these are bounded externally by the ectoderm. The cells of the mesentoderm vary in size, are irregular in shape, and all of them are heavily laden with yolk. Several larger rounded cells stand out rather clearly among the more irregular surrounding cells. One of the most cranial of these is shown in figure 11. It lies directly under the ectoderm, in that part of the mesentoderm which later forms the mesoderm. Many other cells similar to this one are found farther caudad. That these are germ cells is shown by their later history, and the posterior region of the mesentoderm may therefore be considered a region for the proliferation of these cells. The shaded area in figure 5 shows in a diagrammatic way the relative position of the germ cells at this stage.

b. Embryo 238 hours old (fig. 6). A sketch of an embryo 238 hours old, about one day before hatching, is shown in figure 6, and a section along the line *ab* is shown in figure 12. In the latter figure three spherical germ cells, structurally identical with those of the preceding stage, are seen in the lateral portions of the mesoderm which have recently separated from the entoderm. Other germ cells may be recognized caudad of this section and also considerably farther craniad. The relative position of the cells in the whole embryo is shown by the shaded area in figure 6, and it will be seen that they extend much farther craniad than in the preceding stage. They are not arranged in regular groups in relation to the body somites, but form irregular bands, one on each side of the body. At this stage the dorsal part of the mesoderm has separated from the entoderm along its whole length, but its lateral plates are still continuous ventrally with the entoderm except along the cranial part of the germ-cell region, and craniad of it where a complete separation seems to have taken place, but even here one cannot be absolutely certain that the two germ layers are not continuous ventrally. Each lateral plate becomes thinner along the side of the body and terminates ventrally in a sharp edge, but the separation of this edge from the entoderm is not distinct. This makes it very difficult to determine whether the mesoderm extends ventrad at the expense of the entoderm or by independent growth.

In the caudal region of the body the germ cells are large and spherical and stand out clearly among the apparently smaller and more irregular-shaped cells of the yolk entoderm, as shown in figure 13, a section from the region *cd* of figure 6. Farther craniad the germ cells are more irregular in shape due to pressure from surrounding cells. No structural difference could be found between the germ cells and the large yolk-bearing cells of the entoderm, except that the former have more definite outlines. It is probable that even this difference is the result of location rather than of any inherent difference in structure. This suggests the possibility that any of the yolk-bearing cells of the mesentoderm which are so situated that they have a chance to get into the mesoderm at the time it separates from the entoderm may become germ cells. Another probability is that the germ cells are segregated in an earlier stage and that many more are produced in the early development of the embryo than can be included in the mesoderm when it separates. In this case all of the germ cells which remain in the entoderm probably degenerate in situ or are thrown off bodily into the lumen of the intestine. Later some evidence for this will be presented.

*c.* Larva 274 hours old (fig. 7). The larva of this stage has just broken out of the egg membrane and the anterior portion of the body has straightened out, as shown in figure 7. The caudal region, however, which includes most of the yolk, still forms a right angle with the cranial region. The position of the germ cells from three different regions is shown in figures 14, 15, and 16, taken from the parts of the larvae indicated by the lines *ab*, *cd*, and *ef*, respectively. At this stage the mesoderm extends farther ventrad than in the preceding stage. The germ cells lie in the nephrotome region, either ventrad or latero-ventrad of the newly formed pronephric ducts. They are much more numerous than in the preceding stage, and sometimes they lie so close together that in every section two or more cells are found. The absence of mitotic figures and the uniform size of the germ cells indicate that the increase in number is not due to any division of the cells, but to the fact that more and more germ cells are being included in the mesoderm as

its separation from the entoderm extends caudad. There is no indication at this or at any other stage that the germ cells are segmentally arranged. They form two bands which are separated caudally, but converge cranially. The most cranial cells, although older in the sense that they were first to be included in the mesoderm as it became separated from the entoderm, are apparently not different from the posterior cells which were included much later.

*d.* Larva 286½ hours. This stage, shown in section in figure 18, is somewhat more advanced than the preceding. The mesodermic somites in the anterior region have become differentiated into a muscle plate, a dermal plate, and a sclerotome. The pronephric ducts lie in the regions laterad of the muscle plates. Between them and the yolk entoderm, and sometimes indenting the latter, are the large yolk-laden germ cells. They are of the same size and structure as the cells of the preceding stage. Occasionally the cells are found in groups, but no mitosis has ever been observed in the germ cells of this period, and this makes it probable that the cell aggregations are the result of a slight amount of migration or of several cells being separated from the entoderm at the same place. Due to a pressure from surrounding cells, many of the germ cells have lost their rounded appearance at this stage. The bands of germ cells of the two sides approach each other more closely cranially than in the preceding stage, but caudally they still lie far apart.

*e.* Larva 299½ hours (fig. 8). The larvae of this stage have increased considerably in length, but the caudal region is still loaded with yolk and remains perpendicular to the rest of the body (fig. 8). Figure 17 represents a section through the posterior part of the body, from the region indicated by the line *ab* in figure 8. In this section four germ cells are scattered along the lateral plates of the mesoderm. A section near the cranial end of the germ-cell area is shown in figure 19. This contains three germ cells which now have reached a position mediad of the pronephric ducts. Not only do the entoderm and mesoderm lie so close against each other that it is difficult in some places to see the line of separation, but the germ cells often lie in little



depressions in the entoderm formed by the pressure of the germ cells against it. The result is that in certain sections the germ cells appear to be still in the entoderm. Such sections of this late stage examined without knowledge of the previous history of the cells might lead one to believe that they were migrating from the entoderm into the mesoderm. But there is no reason for believing that such a belated migration takes place in the lamprey, for other sections show that practically all the mesoderm has separated from the entoderm at this stage and that all the germ cells which are destined to become functional now lie in the mesoderm. Their position is about the same as in the preceding stage. They have not yet reached the midline cranially, while caudally they are scattered along the lateral plates of the mesoderm so that at the very extreme caudal end they are still found near the midventral line of the gut entoderm.

In later stages germ cells which lie in the lateral plates, far removed from their final destination, are often found in various stages of disintegration. It is also likely that many prospective germ cells never reach the mesoderm, but remain in the gut entoderm either to degenerate in situ or to be thrown off.

*f.* Larva 320 hours old. A larva of this stage is considerably longer than that of the preceding stage. The caudal part of the body is still slightly curved. Cranially the germ cells lie between the dorsal aorta and the pronephric ducts; caudally they lie ventrad or laterad of the ducts. The germ cells are in all respects similar to those of the preceding stage.

*g.* Larva 359½ hours old (fig. 9). The body of a larva of this stage is almost straight (fig. 9). The germ cells, two of which are shown in figure 20 are nearer the middorsal line than before, have lost their rounded contours, and are flattened between the gut entoderm and the pronephric ducts, dorsal aorta, and intervening mesenchyme. The germ cells are still filled with yolk globules and there is no indication that mitosis is taking place. The nucleus is usually eccentric and contains two deeply staining nucleoli, besides scattered chromatin granules. Each germ cell is surrounded by a number of flattened mesoderm cells. Figure 20 shows one germ cell cut through the nucleus and another cut

along one side. The two germ-gland anlagen have not yet fused at any place along the midline, although they approach each other very closely at their cranial ends.

*h.* Larva  $373\frac{1}{2}$  hours old. The germ-gland anlagen extend farther forward than in the preceding stages. Since the large, inert, yolk-bearing cells are very poorly adapted for independent migration, it is probable that their movement craniad as well as mediad is due, at least in part, to the mechanical shifting of the parts surrounding them. A coelom had formed in the cranial portion of the mesoderm and is also forming in the caudal region in front of the anal opening. In the middle portion, however, no body cavity is yet formed. The gut is loaded with yolk and is surrounded by mesoderm. In this mesoderm, on the dorsal side of the intestine, the yolk-laden germ cells occur, sometimes singly and sometimes in groups. Cranially, the two lateral germ-gland anlagen are well defined and practically come together. The cells are greatly flattened dorsoventrally by the pressure of the surrounding tissues. Caudally the germ cells are scattered along the whole lateral plate. In a cross-section from the caudal region slightly craniad of the anal opening, a large germ cell (fig. 21) was found lying in what may be considered the ventral mesentery. It is highly probable that a germ cell so situated will never become functional.

*i.* Larva  $429\frac{1}{2}$  hours old. The posterior cardinal veins have appeared at this stage and the germ cells lie ventrad of these cranially (fig. 22). As the coelomic cavity is being formed by a splitting of the mesoderm, the germ cells become included in the somatic portion (fig. 23).

*j.* Larva  $478\frac{1}{2}$  hours old ( $4\frac{7}{8}$  mm. long). At this stage the germ-gland anlagen have fused cranially (fig. 24); caudally they still remain apart. The cardinal veins have increased in size and now lie dorsomedial of the pronephric ducts. Cranially the mesenchymal tissue has increased greatly in amount in the region in which the germ cells are found. It fills a considerable space between the germ cells, the dorsal aorta, and the cardinal veins. The germ cells are still flattened against the gut ventrally. They still retain their embryonic structure and are not dividing.

*k.* Larva  $538\frac{1}{2}$  hours ( $5\frac{5}{8}$  mm. long) (fig. 26). In the caudal region of the body the germ cells still retain their embryonic form. They are apparently not yet able to reach their final median position on account of the large amount of yolk in the entoderm of this region. Cranially, on the other hand, the yolk in the entoderm is being absorbed so that more space is left for the germ cells, and in consequence they shift their position toward the midline. As a result, the two bands of germ cells are now arranged in the form of a V with the apex pointing cranial. With the release of pressure and with the assumption of a median position, the anterior cells begin to show signs of activity. The yolk globules in many cells have lost their sharp contours and often appear fragmented (fig. 25). Sometimes they are absent from certain parts of the cells. The cytoplasm, hitherto clear, now has a granular appearance. The chromatin material in the nucleus now stains more deeply, and often chromatin-like granules are found in the cytoplasm surrounding the nucleus. It appears probable that there is at this time an active interchange of material between the nucleus and the cytoplasm. No mitoses were observed, however, for a long time subsequent to this stage.

*l.* Larva 647 hours. At this stage the coelomic cavities have formed by a splitting of the lateral plates throughout their length, and the cavities of the two sides have fused and nearly the whole of the dorsal mesentery has disappeared. The germ cells are included in the somatic layer of the mesoderm. Most of them now lie along the dorsal midline directly below the dorsal aorta, but are spread out over a considerable area on each side of it. All have disappeared from the caudal region. No germinal fold is yet present. Some of the germ cells have lost the greater part of their yolk. None of the cells were found in mitosis.

*m.* Larva  $902\frac{1}{2}$  hours. A few germ cells along the posterior portion of the body cavity still retain some yolk, but the great majority of them are now free from it. As compared with the mesodermal cells of the same region, they may be described as large spherical cells with large spherical nuclei each with two large nucleoli. No cells were found in mitosis. Sometimes

two or more cells are so grouped as to suggest that they are derived from one cell by division; but since no mitoses are observed, the grouping is probably the result of a migration of the cells. The larvae of this stage have begun to feed. An examination of larvae between this and the former stages shows that they begin to feed when they are about 7 mm. long, although a great deal of yolk is still present in the intestinal wall.

*n.* Larva 10 mm. long (June 22). This larva was obtained from the creek on June 22nd and is in the neighborhood of seventy days old. The yolk is now all absorbed from the intestinal wall and the lumen of the digestive tract is full of diatoms and other organisms upon which the larvae feed. The germ-gland anlagen are in the middle two-thirds of the coelom, but are absent from its cranial and caudal parts. In later stages, when the cells begin to increase in number by division, their range is extended both cranial and caudal. The germ cells are irregularly distributed along the anlagen with no indication of a segmental arrangement. They lie in the mesenchyme on the ventral side of the dorsal aorta and close against the peritoneum, which consists of very flat epithelial cells (fig. 29). Some of the germ cells may project slightly into the coelom, but these projecting cells do not yet form a continuous germ fold. Although the germ cells may lie against the peritoneum, they never form a part of it. They may be distinguished from the epithelial cells and other cells of the same region by their larger size and spherical shape; by their large spherical nuclei, each containing two large nucleoli, and by their clear transparent cytoplasm (fig. 30). Each is surrounded by flat epithelial cells which are similar to those forming the peritoneum. The germ cells in this stage are absolutely distinct from the cells of the soma, as they appear to be from the time when they are first recognized as germ cells. They have lost all their yolk, but no signs of mitosis could be found.

At this stage the lumen of the intestine is very much enlarged and many of the cells from the walls of the intestine have been set free into the intestinal cavity (fig. 31). This is the case also in much earlier stages (larvae about 7 mm. long), and suggests

that the extruded cells are germ cells which have failed to reach the germ-gland anlage.

*o.* Larva 20 mm. long. This larva is about four months old. The germ fold has now formed (fig. 27) and extends along the dorsal wall of the coelom as a low longitudinal ridge. Some of the germ cells have migrated into the fold, but others are still in the mesenchyme above it. Their position is such that they are practically surrounded by blood-vessels—the posterior cardinal veins laterally and the dorsal aorta above. Besides these vessels, a large number of smaller vessels permeate the tissues around the germ cells. The germ cells occur in groups and in some places long distances intervene between them so that the various groups do not form a continuous band. The germ cells are more numerous than in the preceding stage, and in some cases two are found which apparently are surrounded by a single follicular membrane—an indication of recent division. In most of the cells at this stage, a distinct attraction sphere occurs. It consists of a mass of closely set granules and lies against one side of the nucleus. It may cover as much as one-third of the circumference of the nucleus in each section through the middle of the cell (fig. 28). No distinct centrosome could be found. Besides the attraction sphere, there is in the cytoplasm a spindle-shaped body, the ‘vitelline body’ of King ('08), which is much smaller and is made up of coarser granules than the attraction sphere. In longitudinal section it appears oval, in cross-section, round. Judging from sections through various planes, it is shaped like a spindle which tapers abruptly at both ends. It may occur almost anywhere in the cytoplasm, sometimes near the nucleus but sometimes close under the cell membrane. The origin of this body could not be ascertained. The presence of a centrosphere indicates the beginning of mitotic activity. The cells may now be considered as having passed out of the primary period of rest and entered the period of secondary division. There is yet no indication of sexual differentiation and, in the absence of any characters which distinguish the secondary spermatogonia from the secondary oogonia, the germ cells may be regarded as still indifferent as to sex (table 2).

The germ cells lie against the peritoneum which covers the gland (fig. 30), but they are always separated from the coelom by peritoneal cells and they have never been found to form a part of the peritoneal epithelium. Epithelial cells may be seen, still in part included in the peritoneum, but with processes extending to the germ cells and forming a part of their follicles. These cells occur in all stages of detachment from the peritoneal epithelium and in all stages of inclusion in the follicular membranes of the germ cells. There is no evidence at this stage that the follicular cells are derived from any other source. Both in the peritoneum and in the follicles these cells are distinguishable from the germ cells and mesenchyme cells by their ovoid nuclei, each with one large plasmosome and several smaller chromatin nucleoli, and by their flattened form and indefinite contours. The mesenchyme cells are recognizable by their nearly spherical nuclei, and the germ cells by their large size and spherical nuclei, each with two large plasmosomes. It seems clear from their early history, from the fact that they are at no time seen to be included in the peritoneal epithelium, and from their distinguishing structural characters, that the germ cells are not derivatives of the peritoneal epithelium. It seems equally clear that the follicle cells are derived from this source.

2. *Historical and critical.* a. Invertebrates. A study of the early history of the germ cells in various species of invertebrates has disclosed the fact that they often are segregated during early cleavage. These primordial germ cells are at first distinguished either by the behavior of their chromosomes or by the presence of certain cytoplasmic inclusions. In vertebrates the primordial germ cells are usually not recognizable until the three germ layers are formed, although most investigators of the subject (Beard, Allen, Dodds, Nussbaum, King, Witschi, Rubaschkin, Swift, Tschaschkin, and others) believe they must have been segregated at a much earlier stage. The staunchest adherents of the theory of early segregation (Beard, Allen, Rubaschkin, Witschi Swift, and others) hold that all the definitive germ cells are derived from the primordial germ cells, a conclusion that the theory of the continuity of germ plasm naturally demands.



Others (Abramowicz, Bouin, Kuschakewitsch, Dustin, Firket), while admitting the presence of the primordial germ cells in the early embryo and the possibility that they give rise to definitive reproductive products, still think it probable, and even supported by very strong evidence in some cases, that many of the definitive germ cells are derived from other elements which, strictly speaking, have formed a part of the soma. Opinions vary as to whether these other cells should be considered true somatic elements or simply another type of undifferentiated cells. Child ('06) is convinced that in the cestode, *Moniezia expansa*, the germ cells develop from cells of the parenchymal syncytium which must be regarded as differentiated tissue cells. In his development of the 'theory of dedifferentiation' ('15) he makes the following statement:

In the tapeworm *Moniezia*, for example, the sex cells arise from the parenchyma, and apparently any parenchymal cells which lie within the region involved in the production of sex cells may undergo dedifferentiation and take part in the process. Even the large muscle cells may give rise to testes. . . . In such cases the muscle fiber undergoes degeneration, the vacuoles disappear, and the nucleus begins to divide, apparently at first amitotically (pp. 331-332).

C. W. Hargitt ('06) thinks that the germ cells in *Clava leptostyla* arise in the entoderm, and that it is unlikely, though possible, that these cells may be undifferentiated. In *Campanularia flexuosa*, George T. Hargitt ('13) has found that the egg cell arises in the entoderm by the transformation of single epithelial cells, or from the basal half of divided cells. He concludes: "Therefore the egg cells have come from differentiated body cells (so-called) and there is no differentiation of the germ plasim in the sense that the germ cells are early differentiated and set aside and do not participate in the body functions" (p. 111).

Max Jörgensen ('10) comes to the same conclusion for *Sycon*. He says: "Indessen zeigen mir meine Präparate dass auch eine Entstehung von Oogonien aus Mesodermzellen denkbar und morphologisch nachweisbar ist" (p. 169).

b. Vertebrates. The earliest theory of the origin of germ cells in vertebrates is the 'germinal epithelium theory,' advanced by

Waldeyer in 1870. He found some large spherical cells in the coelomic epithelium on each side of the dorsal mesentery in the early chick embryo, and supposing that they were young stages of eggs he called them 'Ureier'; the epithelium in which they were found he called 'Keimepithel' (germinal epithelium). In 1875 Semper found that both ova and spermatozoa were derived from the so-called Ureier. Waldeyer and his followers believed that these cells were derived directly from the cells of the germinal epithelium, and this idea is held by a few investigators at the present time.

Since the development of the theory of 'early segregation' by Nussbaum ('80) many investigators have worked on the origin of the germ cells in vertebrates. It has been found in most cases in which the early history of the cells has been traced that they do not originate in the coelomic epithelium, among the cells of which they are later found, but that they attain this position after a migration from other parts of the embryo (Woods, Allen, Dodds, King, Witschi, and others). These same investigators have found that the germ cells in very early stages are usually located in the entoderm, from which they migrate to their definitive position in the coelomic epithelium. When first found, they are large yolk-bearing cells of the entoderm and distinguished from the entoderm cells principally by their location.

From numerous investigations on the subject there seems to be no doubt about the existence of the so-called primordial germ cells which are segregated very early in the development of the embryo, but whether or not all, some, or any of the definitive germ cells are derived from these is a question about which there is very little agreement. Rubaschkin ('09, '12) and others hold that the definitive reproductive cells in mammals are derived exclusively from primordial germ cells. Firket ('14) thinks it is possible that a few of the oogonia may be derived from the primordial germ cells, but that most of them are derived from certain cells in the germinal epithelium, which he calls 'gonocytes secondaire.' Kuschakewitsch ('10) believes that in *Rana esculenta* the oogonia are derived from cells in the germinal

epithelium which are descendants of the primordial germ cells; while the spermatogonia are developed from 'Paragonien,' or secondary germ cells, which take their origin in the axial mesenchyme. Von Winiwarter and Sainmont ('09) regard the primordial germ cells in mammals as only temporary structures, which later degenerate. The same conclusion has been reached by Kingery ('17). Von Berenberg-Gossler ('14), from his work on *Lacerta agilis*, comes to the conclusion that the migration of the so-called primordial germ cells from the entoderm is nothing but, "eine späte, sich nach längere Zeit hinziehende Mesodermbildung aus dem Entoderm." He thinks that these cells as well as other mesoderm cells, such as those of the coelomic epithelium, may give rise to the stem cells of the ova and spermatozoa. According to Gatenby ('16), there is in the frog (*Rana temporaria*) and other amphibians an annual transformation of peritoneal cells into germ cells, so that in these forms there can be no talk of a continuity of the definitive germ cells and the primordial germ cells.

Some investigators believe that they have found evidence that the primordial germ cells are segmentally arranged, being derived from segmental portions of the mesoderm (Rückert, Van Wijhe, Dustin, and others). This has been termed the 'gonotome theory.' It is an attempt to homologize the condition found in vertebrates with that found in *Amphioxus* in which the gonads are segmentally arranged from the beginning of their development. According to this theory, the germ cells are derived from mesodermal cells.

Only a few investigators have followed the later history of the primordial germ cells and found that they actually give rise to definitive germ cells. Among these are Witschi ('14) for the frog, King ('08) for the toad, and Swift ('14, '16) for the chick. Many supporters of the theory of early segregation have studied only the early embryonic stages and have assumed that definitive germ cells originate from no other source than the primordial germ cells.

From the above it is clear that there exists at the present time a great diversity of opinion concerning the origin of the

TABLE 3

Outline of theories concerning the origin of germ cells in vertebrates. The various stages in the development of the individual are represented by five vertical columns. The dotted lines indicate the germ paths as conceived by the various theories. The different germ layers are represented by horizontal lines. The proximity of the germ paths to each of these lines indicates where the germ cells are supposed to take their origin and where they may be found during the different stages of development. In the sixth vertical column a brief summary of each theory is given

1	2	3	4	5	6
Blastoderm	Gastrula	Three Germ Layers	Embryo	Adult	
	Ectoderm				Theory of early segregation. Rubaschkin '12 and others.
.....	.....	Mesoderm	.....	.....	
	Mes-entoderm	Entoderm			
	Ectoderm				Germinal epithelium theory and Gonotome theory. Waldeyer '70, Rückert '68, and others.
	.....	Mesoderm	.....	.....	
	Mes-entoderm	Entoderm			
	Ectoderm				Oogonia derived from primordial germ cells, spermatogonia from mesoderm cells (Paragonia). Kuschakewitsch '10.
.....	.....	Mesoderm	.....	.....	
	Mes-entoderm	Entoderm			
	Ectoderm				Primordial germ cells degenerate; secondary germ cells derived from mesoderm. Von Winiwarter and Sainmont '09.
.....	.....	Mesoderm	.....	.....	
	Mes-entoderm	Entoderm			
	Ectoderm				Primordial germ cells may form definitive germ cells but these may also come from mesoderm cells. Von Berenberg-Gossler '14.
.....	.....	Mesoderm	.....	.....	
	Mes-entoderm	Entoderm			
	Ectoderm				Primordial germ cells may give rise to a few oogonia but most of the definitive germ cells are derived from mesoderm cells. Firket '14.
.....	.....	Mesoderm	.....	.....	
	Mes-entoderm	Entoderm			

reproductive cells in animals. Table 3 presents a summary of the various theories relating to the origin of the germ cells in vertebrates.

For one who adheres closely to the germ-plasm theory it is hard to conceive of the germ cells as coming from any other cells than the early segregated embryonic cells which have had no part in the building up of the body. Some who believe in the theory of early segregation maintain that, even when the germ cells appear to arise from so-called somatic elements, these are in the strict sense not somatic cells, but cells that have maintained their embryonic structure and have not specialized in any given direction. There is, of course, no direct evidence for this. In many forms, it is true, the germ cells seem to be segregated very late in the life of the individual. This is apparently true of annelids and flatworms among animals, and it seems to be true of all plants. There are no investigators of germ cells in vertebrates who maintain that they come from highly differentiated somatic cells, such as muscle cells, as observed by Child ('06) in *Moniezia*.

The various theories concerning the origin of germ cells in vertebrates have now been stated. Below is a partial list of the most important contributions on the subject, each followed by a brief statement of the conclusions which the various investigators have reached. The references have been arranged chronologically for the various groups of vertebrates.

AUTHOR	YEAR	SPECIES	RESULTS AND CONCLUSIONS
<i>Cyclostomes</i>			
Goette	1890	Petromyzon fluviatilis	Germ cells derived from the mesoderm
Wheeler	1899	Petromyzon planeri	Germ cells derived from blastoderm cells
Beard	1902	Petromyzon planeri	Germ cells are early segmentation cells. Their number is 2 <sup>8</sup> -1
<i>Elasmobranchs</i>			
Semper	1875	Plagiostomes	Germ cells are derived from the coelomic epithelium
Balfour	1876 1877	Scyllium, Pristiurus	Germ cells are probably derived from the mesoderm. They may have been introduced from elsewhere
Rückert	1888	Pristiurus	Germ cells are derived from segmental mesoderm cells. Gonotome theory
Van Wijhe	1889	Scyllium, Pristiurus	Germ cells are derived from segmental mesoderm cells. Gonotome theory
Beard	1900 1902	Raja batis, Pristiurus	Germ cells are derived from early segmentation cells
Woods	1902	Squalus acanthias	Early segregated germ cells are first found in the entoderm
<i>Ganoids</i>			
Allen	1909	Amia Lepidosteus	Germ cells are segregated early and are first found in the entoderm
<i>Teleosts</i>			
Nussbaum	1880	Trout	Germ cells are segregated early and are first seen in the region of the germ gland, but they are not derived from the mesoderm
MacLeod	1881	Hippocampus, Belone	Germ cells are derived from the germinal epithelium
Hoffmann	1886	Salmon	Germ cells are derived from peritoneal cells
Eigenmann	1891 1896	Micrometrus aggregatus	Germ cells are segmentation cells from about the fifth generation
Böhi	1904	Trout, salmon	Germ cells are derived from cells of the coelomic epithelium



AUTHOR	YEAR	SPECIES	RESULTS AND CONCLUSIONS
<i>Teleosts—Continued</i>			
Federow	1907	<i>Salmo fario</i>	Germ cells are first found in the somatopleure and splanchnopleure
Dodds	1910	<i>Lophius piscatorius</i>	Germ cells are first found in the primary entoderm. They are early segmentation cells
Bachmann	1914	<i>Amiurus nebulosus</i>	Germ cells are first found in the lateral plate of the mesoderm. They are early segregated cells
<i>Urodeles</i>			
Dustin	1907	<i>Triton alpestris</i>	Germ cells are derived from mesoderm cells (gonotome)
Spehl and Polus	1912	Axolotl	The germ cells are derived from mesoderm cells
Schapitz	1912	<i>Amblystoma</i>	The germ cells are derived from mesoderm cells (gonotome)
Abramowicz	1913	<i>Triton</i>	Primary germ cells are derived from the entoderm (early segregation cells). Secondary germ cells are derived from the mesoderm
<i>Anura</i>			
Nussbaum	1880	<i>Rana fusca</i>	Germ cells are first found in the mesoderm, but are early segregated cells
Bouin	1901	<i>Rana temporaria</i>	Germ cells are derived from early segregated cells, and from peritoneal and mesenchyme cells
Allen	1907	<i>Rana pipiens</i>	Germ cells are derived from early segregated cells. They are first found in the entoderm
Dustin	1907	<i>Rana fusca</i>	Germ cells are derived from the lateral plate of the mesoderm (gonotome) and from peritoneal cells
Dustin	1907	<i>Bufo vulgaris</i>	Germ cells are derived from the lateral plates of the mesoderm (gonotome) and from peritoneal cells
King	1908	<i>Bufo lentiginosus</i>	Germ cells are early segregated cells and are first found in the entoderm
Kuschakewitsch	1910	<i>Rana esculenta</i>	Primary germ cells are derived from early segregated cells. Secondary germ cells are derived from the peritoneal epithelium and axial mesenchyme

AUTHOR	YEAR	SPECIES	RESULTS AND CONCLUSIONS
<i>Anura—Continued</i>			
Champy	1913	<i>Rana temporaria</i>	Germ cells are derived from segmental mesoderm cells (gonotome)
Witschi	1914	<i>Rana temporaria</i>	Germ cells are derived from early segregated cells and are first found in the entoderm
Gatenby	1916	<i>Rana temporaria</i>	Germ cells originate periodically from peritoneal cells in adult frogs
<i>Reptiles</i>			
Allen	1906 1907 1911	<i>Chrysemys marginata</i>	Germ cells are first found in the entoderm and are derived from early segregated cells
Jarvis	1908	<i>Phrynosoma cornutum</i>	Germ cells are first found in the entoderm and are derived from early segregated cells
Dustin	1910	<i>Chrysemys</i>	Primitive germ cells are derived from entoderm cells. Secondary germ cells come from peritoneal cells
Von Berenberg-Gossler	1914	<i>Lacerta agilis</i>	So-called primordial germ cells are formed in the entoderm. They are probably not germ cells, but give rise to mesoderm cells. Germ cells are derived from mesoderm
<i>Aves</i>			
Waldeyer	1870	Chick	Ova are formed from cells of the germinal epithelium. Spermatogonia come from cells of the wolffian duct epithelium
Hoffmann	1892	Twelve species of birds	Germ cells are early segregation cells
Nussbaum	1901	Chick	Germ cells are first found in the splanchnopleure, but they are early segregated cells
Rubaschkin	1907	Chick, duck	Germ cells are early segregation cells first found in the splanchnopleure
Tschaschin	1910	Chick	Germ cells are first found in the splanchnopleure. They are early segregated cells
Von Berenberg-Gossler	1912	Chick	The so-called germ cells first found in the splanchnopleure may not be germ cells

AUTHOR	YEAR	SPECIES	RESULTS AND CONCLUSIONS
<i>Aves—Continued</i>			
Firket	1914	Chick	Primordial germ cells may give rise to definitive germ cells, but most of these are derived from the germinal epithelium
Swift	1914 1916	Chick	Germ cells are derived from cells in the germ wall entoderm. They are early segregated cells
<i>Mammals</i>			
Allen	1904	Pig, rabbit	All functional germ cells are derived from the peritoneum
Sainmont	1906	Cat	Primitive ova are present, but are not functional. Definitive germ cells are derived from epithelial cells
Winiwarter and Sainmont	1909	Cat	Primitive ova are present, but are not functional. Definitive germ cells are derived from the germinal epithelium
Rubaschkin	1908 1909 1912	Cat, rabbit Mole, porpoise Guinea pig	Germ cells are derived from early segregated cells and are first found in the entoderm. There is no secondary origin of germ cells

c. Review of work on the early history of the germ cells in lampreys. W. Müller ('75) described the germ glands of young lamprey larvae as median, unpaired thickenings of the peritoneum situated between the bases of the mesonephric bodies and extending along the whole length of the body cavity. At this stage groups of germ cells were found, but sex could not be distinguished.

Goette ('90) found the reproductive cells in larvae of a much earlier stage, corresponding approximately to that represented by my figure 7. He observed that, while most of the cells in the mesodermal plates soon lost their yolk and began to divide, some of the cells retained their yolk and remained undivided. These cells were found in the mesodermal plates on both sides along their thickened median portions directly under or outside of the pronephric ducts and sometimes against the yolk entoderm,

so that it appeared as though they might belong to it. He says, however, "Eine genaue Untersuchung hat mich aber überzeugt dass es ursprüngliche Mesoderm-elemente und nicht etwa vom Darmblatt her eingewanderte Zellen sind" (p. 53).

Wheeler ('99) has given an excellent account of the early development of the germ cells in the lamprey (*Petromyzon planeri*). He recognized the germ cells in the posterior region of embryos as early as my figure 5, the stage in which they were first observed by me in *Entosphenus wilderi*. Wheeler found: "Just laterad to the myotomes a few very large rounded masses of yolk." He described each mass as containing a nucleus and "more or less distinctly marked off from the adjacent entoderm elements." He says further, "These large masses are the primitive reproductive or sex cells. They can hardly be assigned to the mesoderm because their appearance and position are those of entoderm cells in this stage. Still they lie in a portion of the entoderm which becomes mesoderm with the more lateral extension of the latter layer."

Beard ('02), in an attempt to work out a numerical law for the primordial germ cells in animals, says that the number of cells should in each case be  $2^n - 1$ . His theory is that the blastoderm in animals corresponds to the sporophyte in plants, and to it he applies the term 'phorozoon.' After a time one of its cells divides a definite number of times and forms the primordial germ cells. The number of divisions varies according to the species. One of these primordial germ cells is sacrificed to form the embryo so that the actual number of germ cells remaining is in each case  $2^n - 1$ . In the case of the lamprey (*Petromyzon planeri*) Beard finds that  $2^n = 32$  and that therefore in this species the number of primordial germ cells is thirty-one.

Interesting in connection with the description of the early history of the germ cells in the lamprey is an observation made by Kupffer ('90). In the early gastrula of *Petromyzon planeri*, he found, between the ectoderm and the entoderm in the region of the blastopore, certain cells which he called 'teloblast cells.' They were easily distinguished from the yolk cells adjoining them, but their origin was not observed. Kupffer thinks that

the mesoderm in this region develops at the expense of these cells. He says:

Wenn aber das dorsale Mesoderm entstanden ist und bis zum Teloblast reicht, tritt es in dieselbe einige Verbindung damit wie der Neuralstrang und die Chorda, und nachdem die Segmentierung des Mesoderm bis zum Schwanzende fortgeschritten ist, ergänzt sich der jeweilig hinterste Abschnitt des Mesoderm durch Zellen die aus dem Teloblast stammen.

Hatta ('92, '07) describes certain cells as budding off between the ectoderm and the entoderm in the region of the blastopore. These cells he calls the 'peristomal mesoblast.' He could not find any cells that corresponded to Kupffer's teloblast cells. It is possible that Kupffer's teloblast cells and Hatta's peristomal mesoblast cells are identical and that they correspond to the large yolk-laden cells which later become included in the mesoderm and form the germ cells.

3. *Discussion.* a. *Early segregation.* We have seen that the germ cells in *Entosphenus wilderi* may be traced to the large yolk-bearing cells which at first are located in the mesentoderm. This is in agreement with the observations of Wheeler. The history of these cells, previous to their inclusion in the mesoderm is not known. They apparently lie among similar yolk-bearing cells belonging to the entoderm, and it is a question whether or not they are essentially different from these. The germ-plasm theory, as expressed by Weismann, demands a segregation of the germ cells at a very early stage, or their origin, at least, from cells that have never taken any part in the formation of body tissues. In one sense all the yolk-bearing cells of the entoderm may be considered as undifferentiated cells, but only some of these cells which are included in the mesoderm become germ cells. Most of the mesoderm cells, however soon begin to divide, become smaller, lose their yolk, and form various tissues, while the large cells that become germ cells do not change in the least for a very long time. This indicates that they are endowed with certain qualities which distinguish them from the cells that become somatic.

My observations and those of Wheeler ('99) show that the germ cells appear first in the posterior region of the body, probably in a small area around the blastopore. None have been seen to separate from the entoderm very far craniad of this region, so that those found later in the region further craniad gain this position by some form of migration. In *Entosphenus wilderi* the peristomal and the paraxial regions of the mesoderm are continuous and do not differ in structure or in origin as maintained by Hatta ('92, '07). The only distinction found between them is that the mesoblast of the paraxial region is delaminated earlier than that of the peristomal region, but only the peristomal mesoderm carries the germ cells in early stages. In *Entosphenus wilderi* no cells corresponding to Kupffer's teloblast cells were found in this region. These may have been either germ cells or, as he supposed, mesoderm cells.

The condition in the lamprey is in favor of the theory of early segregation. The large yolk-laden cells that are at first found among the other yolk-bearing cells of the entoderm in the caudal region of the body become included in the peristomal mesoderm when it separates from the entoderm by a process of delamination. These cells retain their embryonic structure for a long time after the other elements of the mesoderm have become differentiated. Even after all their yolk has been used up, they remain as large conspicuous cells among the smaller somatic cells of the germ gland. Up to this point the history of these cells has been traced and the later history to a time when they begin to divide. Later it will be seen that there is in *Entosphenus wilderi* no evidence that any other cells take part in the formation of definitive germ cells. What evidence there is in other forms will now be considered.

Two lines of evidence have been advanced for the secondary origin of germ cells from mesodermal elements. The first is that transitional stages have been found between mesodermal cells and true germ cells (Abramowicz, Dustin, Firket, Gatenby, and others), but in most cases the figures which purport to represent this transition are not convincing. The second line of evidence is that advanced by Kuschakewitsch ('10). He found

that in the frog (*Rana esculenta*) all the embryos produced by eggs in which fertilization had been delayed for a certain number of hours were males. He believes that normally oogonia are derived from primordial germ cells which are situated in the germinal epithelium, while the spermatogonia are derived from the axial mesenchyme. When fertilization is delayed no primordial germ cells are produced and all the definitive germ cells come from mesodermal cells which he calls 'Paragonien.' These give rise only to spermatogonia, and the larvae are therefore males. Witschi ('14), who more recently has worked on the development of the germ cells in *Rana temporaria*, finds that in this form there is no secondary origin of germ cells. He says:

So scheinen alle Tatsachen dafür zu sprechen, dass von ihrem frühesten Erscheinen an, die Keimzellen als Gebilde spezifischer Natur zu betrachten sind, welche, wenigstens unter Bedingungen die von normalen nicht sehr abweichen, weder sich in somatische Elemente umwandeln, noch aus solchen durch Umwandlung entstehen können.

Among others who have followed the history of the germ cells and have found no evidence of secondary origin, may be mentioned King ('08) for *Bufo* and Swift ('14, '16) for the chick.

b. Method of migration of the germ cells. Three opinions have been advanced concerning the method by which germ cells reach the germ-gland anlage in vertebrates: 1. Most investigators are inclined to the belief that, by some sort of ameboid movement, there is an active migration of the germ cells from the entoderm to the splanchnic mesoderm, and then through the mesentery into the germ-gland anlage. That such migration exists seems certain in forms in which the germ cells are so late in arriving at their final destination that they lose their yolk before leaving the entoderm.

2. A second theory is, that in some of the lower forms the migration of the germ cells may be accounted for partly by a shift in the position of the somatic tissues around them. In this case the germ cells are considered passive elements which take little or no active part in the migration.

It seems likely that in some forms migration is partly active and partly passive. This is probably true in the lamprey. No

ameboid forms have been observed in the yolk-laden germ cells of the early embryo, and it is not likely that they are capable of independent movement, but it is probable that they are transported to the germ-gland region by a shifting of the tissues during somatic differentiation. In later stages, after all the yolk has disappeared, the germ cells extend farther and farther craniad. In sections of these stages the individual germ cells always have a spherical shape and no pseudopodial processes have been seen to indicate that they migrate independently; yet it seems probable that in life there may be a slight amount of migration in this way. Individual germ cells and cysts are usually not in contact, so that it is a little difficult to conceive of these shifting to a more cranial position as a result of the pressure of the cells or cysts against one another as they increase in number. Since the follicle cells show ameboid processes, it may be that the germ cells are carried along by a movement of these.

Some of the germ cells in the lamprey, as well as in other forms, never reach the germ gland, but remain in the entoderm or some other part of the body. Some of these cells may divide and form cell nests in other organs. Such cell nests have been found in the lamprey, both in the fat body and in the median and lateral portions of the mesonephros (figs. 32, 33). The fate of these cell nests is not known, but they probably degenerate.

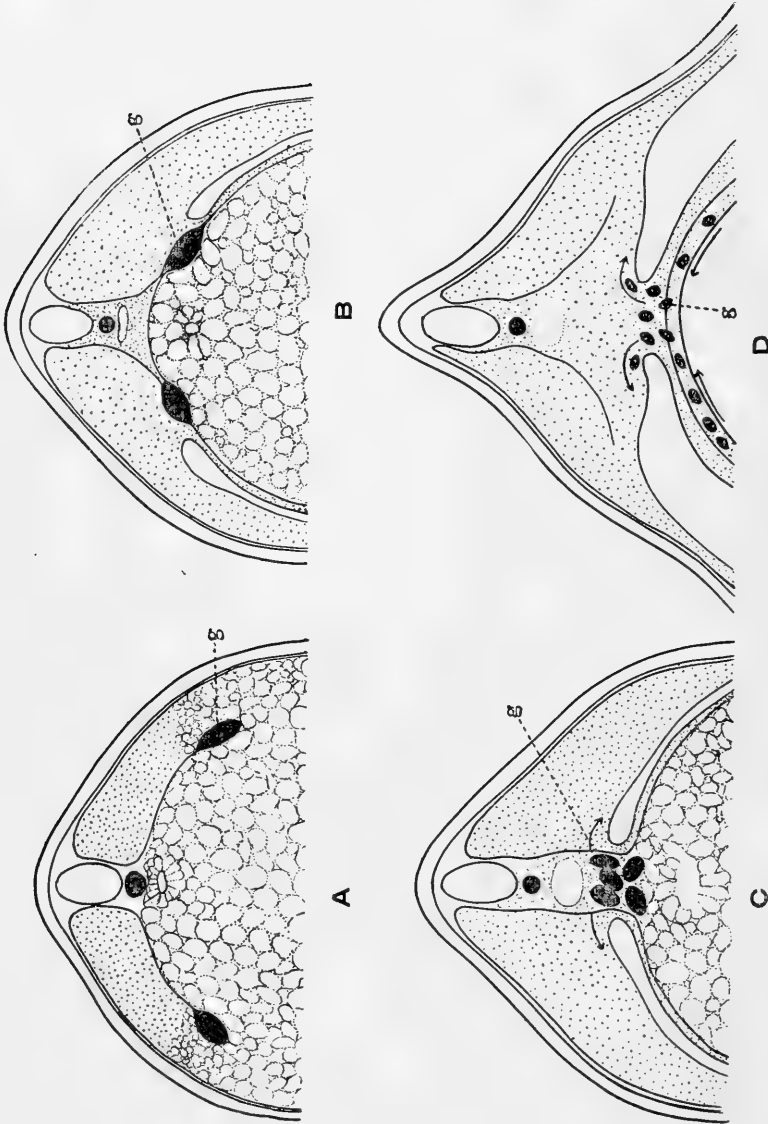
3. A third method of migration appears to have been observed by Swift ('14) in the chick embryo. He says that the large yolk-laden germ cells, which are first found in the germ-wall entoderm, are taken up by the blood-vessels and carried by the blood-stream to the germ-gland region. In fact, the germ cells may be carried to any part of the body, but it is only in the germ-gland region that they migrate out of the blood-vessels. Swift ('16) has followed the later history of these cells and has come to the conclusion that they give rise to the definitive germ cells. Von Berenberg-Gossler ('14), who worked on the early germ cells in duck embryos, also found cells similar to those described by Swift, but he expresses a doubt as to whether or not they are germ cells. He says: "Alles in allem bin ich der Ansicht, dass das ganze Verhalten dieser Zellen in höherem Grade davor warnt sie für Keimbahnzellen zu halten" (p. 261).



A study of the methods by means of which germ cells migrate from the entoderm into the mesoderm in the various groups of vertebrates, and of the time at which this migration takes place, shows that these groups may be arranged in an interesting phylogenetic series as represented in the following diagrams (text figures A, B, C, D).

In the lamprey (text figure A) the germ cells are shown as being included in the mesoderm at the time when it becomes separated from the entoderm. In Triton (text figure B), according to Abramowicz ('13), the germ cells migrate into the mesoderm before the dorsal mesentery is formed, but much later than in the case of the lamprey. In the frog, according to Allen and others, the germ cells are separated from the yolk entoderm at the time the dorsal mesentery is formed, as shown in text figure C. In each of the first two forms, therefore, the original germ-cell anlage is double, while in the latter it appears to be unpaired at first and to lie along the middorsal line above the gut entoderm. In the lamprey, although the original anlage is paired, the germ gland later becomes single by a fusion of the two parts on the ventral side of the dorsal aorta. In Triton the paired anlagen fuse, but later they become paired again, and in the frog the original unpaired anlage becomes paired. In reptiles and mammals the germ cells usually separate from the entoderm much later than in the other three forms, often after they have lost all their yolk. Then migration follows: first into the splanchnic mesoderm, then through the dorsal mesentery, and from there to the germ-gland anlage on each side (text figure D).

c. Relation of germ cells to body cells. In the later embryonic stages of many forms, when the germ cells have reached their final destination in the region of the germ gland, they lie among the peritoneal cells covering the definitive germ-gland anlage and apparently form a part of the peritoneal membrane. This fact gave rise to the idea that the cells were derived from the peritoneal cells, so that this portion of the peritoneum became known as the germinal epithelium. In the lamprey there is, strictly speaking, no germinal epithelium since the germ cells never form a part of the peritoneal membrane, but are independent elements



Text figures A, B, C, and D. A phylogenetic series showing by what method the germ cells of various vertebrate embryos attain their position in the mesoderm. A, lamprey; B, triton; C, frog; D, reptiles and mammals. (For description see text.)

situated in the mesenchyme dorsal to the peritoneum. Sometimes the germ cells may lie so pressed against the peritoneum that its cells become greatly flattened, but the continuity of the peritoneal epithelium is apparently never broken. I have found no evidence that any germ cells are formed out of peritoneal cells at any stage.

*d.* Number of germ cells. No evidence was found that division takes place in the germ cells of the lamprey before the larva is about 20 mm. long. The germ cells are few in number, and, as the young larva continues to grow, they become more and more scattered along the whole length of the body cavity, so that in following a series of sections they are often found far apart. In a larva about 8 mm. long, in which the germ cells stood out with great clearness, thirty-six were counted. These were distributed through 153 10- $\mu$  sections or along 1.53 mm. of the body length, as shown in figure 10. The distribution of the cells by sections was as follows (table, p. 40):

It will be seen that at this time no germ cells are found in the caudal region of the body for about 0.58 mm. in front of the anal opening, and cranially they do not extend any farther than to about the middle of the coelomic cavity.

The number of germ cells found in this larva comes very near that found by Beard ('02) in larvae of *Petromyzon planeri*, but it can hardly be cited as supporting his theory, especially since it is known that many of the germ cells never reach the germ-gland region. It is highly doubtful if the number of germ cells segregated in the development of the embryo is constant, and it certainly seems probable that the number of germ cells that reach the germ gland must vary greatly in different individuals. It would be futile, therefore, to form any conclusions concerning the number of primordial germ cells from a count of those that reach the germ gland.

*4. Conclusions and summary.* Evidence presented supports the theory that germ cells are segregated very early in the development of *Entosphenus wilderi*. These primordial germ cells are first found in the yolk entoderm and become included in the mesoderm when it separates from the entoderm. The germ cells

occur at first only in the posterior region of the body, but they gain a more cranial position with the growth and development of the larvae. The movement of the germ cells craniad is probably

SECTION CRANIAD OF ANUS	NUMBER OF GERM CELLS
58	1
61	1
68	1
76	1
80	1
84	1
86	1
92	1
98	1
102	1
104	1
105	1
106	1
108	1
111	1
120	1
122	1
129	2
135	1
144	1
148	1
155	1
156	1
157	1
158	1
159	1
165	1
166	1
174	1
177	1
182	2
197	1
207	1
211	1
Total.....	36

a passive one in early stages and results from a shifting of the tissues during development. In later stages, after the cells have lost their yolk, migration is probably due partly to an active

movement of the cells. The original double anlage of the germ gland is reduced to a single median gland in later stages, due to a fusion of the two parts along the midline. This fusion begins at the cranial end of the gland. The germ cells which do not reach the germ gland probably degenerate. Some of them form extraregional cell nests, the fate of which is not known. There is no division of the germ cells before the larvae have reached a length of about 2 cm. Long before this all the yolk has been absorbed. Granules staining like chromatin are found in the cytoplasm around the nucleus when the yolk begins to disappear in the cells. When the yolk has been absorbed, two cytoplasmic bodies are found, one a spindle-shaped, granular vitelline body, the other an astrosphere (yolk nucleus). The origin of the vitelline body was not ascertained. The number of the primordial germ cells is small. In one count thirty-six were found. There is no indication during the early stages that the germ cells are derived from any other source than the early segregated cells.

### *C. Period of secondary division*

After the primary period of rest, already described, during which the germ cells do not divide, they enter upon a period of division (table 2). In the following pages the changes are described which take place in the germ gland during this period, which begins when the larva is about 20 mm. long.

1. *Observations on Entosphenus wilderi.* a. Larva 21.5 mm. long, August 23 (fig. 34). In this larva, somewhat more than four months old, the germ-gland fold is still interrupted. While many single germ cells are found and many cysts of less than eight cells, there are a few of from eight to sixteen cells, and the latter number is rarely exceeded in this stage. Most of the germ cells are in the resting stage, but a few are in various phases of mitosis.

The gland is 2.5 mm. long and extends from a point near the middle of the mesonephros, about 2.5 mm. back of the cranial end of the coelom, to a point about 2 mm. in front of the anal opening. Its position, therefore, coincides about with the

middle third of the coelomic cavity and not with its whole length as in older larvae.

Where the coelomic epithelium covers the germ gland it is thicker than elsewhere and its nuclei are much more numerous. These nuclei are ovoid in shape and are usually so oriented that their long axes are parallel to the long axis of the body and to one another. Each contains, besides a chromatin network, a large nucleolus, usually near its center (fig. 35). Many of the larger chromatin granules lie against the inner surface of the nuclear membrane. No membranes are visible between the cells of the peritoneum, but cell limits are indicated by the lessened thickness of the cytoplasm between the nuclei. All the cells of the germinal peritoneum are similar and in no case was the peritoneal membrane found to be interrupted so that the germ cells might be said to form a part of it. Evidence has already been presented to show that these two kinds of cells are of different origin and that there is no genetic relationship between them. That the follicle cells are derived from peritoneal cells, as in earlier stages, is indicated by the similarity in structure of their nuclei and by the fact that in various places peritoneal cells are migrating inward from the peritoneal covering of the gland to take part in the formation of follicles around the cysts and individual germ cells. When a germ cell or cyst lies against the peritoneum, the cells of the latter are usually heaped up around it in such a way as to suggest that they will eventually enclose it altogether. Some of the peritoneal cells also migrate into the interior of the gland without at first coming into relation with germ cells. This usually happens where the peritoneal membrane is indented, in which case the cells separate from the apex of the indented portion (fig. 35). Whether or not all these cells finally attach themselves to germ cells and form follicles for them is not known, although in some cases they seem to become associated with the more deeply situated germ cells. For a time at least, they make up most of the stroma of the gland, although part of the latter enters the gland from the mesenchyme dorsal to it. The nuclei of these few mesenchymal stroma cells may be distinguished from the peritoneal

and follicle cells by their smaller size, structure, and rounded form. The cytoplasm ramifies in all directions so as to form a loose parenchymatous structure (fig. 36). Blood-vessels enter the gland from above with the mesenchyme and fill practically the whole of some sections. The endothelium of these blood-vessels consists of the usual flattened cells containing much flattened nuclei.

The germ cells remain as distinct elements with more or less clear cytoplasm, usually without observable limiting membranes, and with large rounded nuclei. In each nucleus there are, as before, two deeply staining nucleoli and a distinct chromatin network. One has no difficulty in distinguishing the germ cells from the somatic elements of the gland by their nuclear structure and the much greater size of both nuclei and cell bodies. No transitional stages were found between the germ cells and the somatic cells of the gland, and no indication of the transformation of somatic elements into germ cells or germ cells into somatic cells. The number of the germ cells is increased rapidly during this stage, but so many of them are in mitosis that the increase in their number may easily be accounted for without supposing that new germ cells are being formed out of surrounding tissue cells.

b. Larva 25 mm. long (fig. 37). The structure of the germ gland in a larva of this stage is similar to that of the preceding stage, except that, owing to the enlargement of the gland without corresponding increase in the number of germ cells and cysts, these are more scattered than in the earlier stages. In many sections not a single germ cell occurs. In some sections both germ cells and blood-vessels are absent and the peritoneum encloses only a loose stroma with greatly scattered nuclei.

The gland is now suspended from the middorsal line of the coelom by a broad mesentery, in which germ cells are present, while some are still found even in the mesenchyme above it. The germ cells and cysts often lie against the peritoneum and new follicle cells are being formed continually by division and migration of peritoneal cells.

c. Larva 27.5 mm. long. Although the histological features of the gland have not changed from those of the preceding stage, this particular larva was selected for study because certain cytological structures in the germ cells are more clearly shown. One of these is the centrosphere, which differs from that of the 20-mm. larva, in which it was last referred to, in that it shows a distinct centrosome. The vitelline body, as variable in position as in the 20-mm. larva, is visible. It is now more compact and stains black in sections treated with iron haematoxylin. In favorable sections the outline of each individual germ cell may be faintly seen in the cysts (fig. 38). The germ cells are in various phases of mitosis, but most of them are in the resting phase, as in previous stages. Those of a cyst do not divide simultaneously, but several cells within the same cyst may be in different phases of division, while others are in a resting condition (figs. 39 and 67). The cysts range from two to over thirty cells and there are a great number of isolated cells.

d. Larva 30 mm. long. In most of the larvae of this stage the germ glands are similar to those of the preceding stage. In one (larva no. 622) a large number of germ cells were in mitosis. The products of the division of an isolated germ cell may become enclosed, each in its own follicle, or they may remain together as a part of a nest of cells enclosed in a common follicle. The follicle cells of such a nest often stretch into the spaces between the germ cells, while other follicle cells, recognizable by the form and structure of their nuclei, are detached from the follicular membrane and lie free among the enclosed germ cells. Follicle cells occur in all stages of the process of detaching themselves from the follicles and also from the peritoneum, and of penetrating between the germ cells of the nests (fig. 40). In this way, as the germ cells increase in number, old cell nests are broken up and new ones formed, so that in this larva very few nests remain which have more than eight cells. The many follicle cells necessary to enclose the increasing number of nests are probably produced by proliferation of cells already in the follicles as well as of those in the peritoneal epithelium. If this be so, the mitoses must take place very rapidly, for dividing nuclei in either fol-



lie or peritoneal cells are rare. There is no indication that follicle cells are derived from germ cells. If this were the case, transitional stages should be found which might be detected by nuclei of intermediate form and structure, but such have never been found.

*e.* Larvae 34 to 35 mm. long. In the gonad of this stage many single germ cells, as well as many cysts, occur ranging in size from two cells to one hundred or over. The cysts are surrounded, as before, with follicular cells. In many of them the germ cells are in a resting condition, but in some they are in mitosis. Sometimes cell nests, identical with those of the germ gland, occur in abnormal positions. One of these from the fat body is shown in figure 32. Some of the germ cells at this period have entered the synapsis phase, while others have transformed into growing oocytes, the first visible sex-distinguishing character.

2. *Historical review and theoretical discussion.* Wilhelm Müller ('75) studied the early stages of development in *Petromyzon fluviatilis* and *P. planeri*. He gives an account of the reproductive system in a larva 35 mm. long, but the earlier stages were apparently not studied by him. At this stage the sexes could not be distinguished. Oocytes were first observed in larvae 50 mm. long, and in larvae 65 mm. long the ovary and testis were fully differentiated.

Lubosch ('03) found the first anlage of the sex gland in larvae of *Petromyzon planeri* 18 mm. long. He found two types of cells in the gland, the follicle cells and the germ cells, and he believed that the peritoneum was the source from which both were derived. In a slightly later stage he describes a germ ridge as being formed, into which connective tissue and blood-vessels descend from the region dorsal to the gonad. He observed that peritoneal cells migrate inward to form the follicles surrounding the germ cells. He found that the glands remained undifferentiated until the larvae are about 4 cm. long when the ovaries could be distinguished from the male glands.

No further literature exists on this stage in the development of the germ gland in the lamprey.

In the lamprey, according to evidence presented above, most of the somatic part of the germ gland comes from the peritoneum. This is certainly true of the follicular epithelium and a considerable portion of the interfollicular tissue. On the other hand, some mesenchyme is included in the gland fold as it is formed and some is carried in later by the blood-vessels.

Whether or not the follicular cells take any active part in nourishing the germ cells in the early stages is not known. The only case of a true nurse cell observed in the germ gland of lower vertebrates in early stages is that described by Kuschakewitsch ('10). He found what he considered to be nurse cells in frog larvae which developed from his so-called 'Spätbefruchteten' eggs. They were distinguished by greatly concentrated nuclei and by cytoplasm filled with granules which he thinks are chromidia. These nurse cells were not, according to him, themselves used as food for the neighboring germ cells, but served as a source of certain ferments which were useful in preparing nourishment for the cells. He says concerning them: "Es handelt sich aber um eine Sezernierung von Fermenten, welche es bewirken dass die in der Keimanlage zirkulierenden Nährstoffe von den Ampulnelementen besser ausgenützt werden können."

A proliferation of peritoneal cells to form follicle cells has been observed by other investigators in various groups of vertebrates other than the cyclostomes. In the frog it has been observed by Bouin ('01), Dustin ('07), Kuschakewitsch ('10), and others. Bouin believes that these cells take part in the formation of germ cells as well as follicle cells. Kuschakewitsch does not believe that they take part to any great extent in the formation of germ cells. Dustin states that he observed the transformation of ordinary peritoneal cells into germ cells in the turtle (*Chrysemys*). Allen ('06), however, working on the same form, found no evidence of such transformation. In *Triton*, Abramowicz ('13) observed the proliferation of peritoneal cells in the germ gland, and believes that these cells give rise to follicle cells and interfollicular tissue as well as to germ cells, and to the cells of the sex cords. King ('08) thinks that in *Bufo lentiginosus* the peritoneal cells give rise to all the elements of the sex gland with the exception of the germ cells.

The germ cells of the lamprey have not been seen to divide except by mitosis. The nuclei of the cells are always spherical and never have the lobulated appearance which seems to be characteristic of the nuclei of the germ cells of amphibians. Several investigators (Vom Rath, '91; Meves, '91; McGregor, '99) have recorded amitosis as taking place in the germ cells of the latter group, but it is possible that the appearance of amitosis comes from sections through lobulated nuclei. Recently Macklin ('16) has made a study of apparent amitotic phenomena in heart cells of chick embryos growing in vitro, and has found that nuclei divide by bilateral and unilateral constriction, but that afterward the parts of the nuclei recombine and divide by normal mitosis. It may be that in some animals similar processes take place in the germ cells.

In the lamprey the cells in the same cyst do not always divide simultaneously, though generally, if the cells in a cyst are dividing, most of them are in one phase or another of mitosis. At the same time the cells in neighboring cysts may all be in a resting stage. Since the germ cells do not multiply very rapidly, there is a considerable period of rest between successive simultaneous divisions in a cyst. The synchronous division of the cells of a cyst may be due to their close relationship, all being derived from one cell and having been subjected to similar environmental influences, or to some stimuli from outside sources, the effect of which is limited to a single cyst. King ('08) found that in *Bufo* the cells of a cyst did not divide simultaneously. According to Jørgensen ('10), this is also true in *Proteus*. Bouin ('01), however, describes the cells of a cyst as dividing simultaneously in the frog. It seems likely that there may be considerable variation in this respect in different forms and even in the same species.

Since, in the lamprey, most of the cysts are broken up from time to time by the inward migration of follicular cells, it is impossible to say whether or not there is a constant number of divisions of the indifferent germ cells. In some cases the cysts become very large and contain hundreds of cells, but it is not certain that only these came from one primordial germ cell. In most, if not in all cases, each primordial germ cell gives rise to many

cysts, probably to an indefinite number. In *Bufo* it appears that the cysts do not break up, for, according to King ('08), all the cells of a cyst are descendants of one primary oogonium in the female, and the cyst wall is formed out of the original follicle cells which surrounded the primary oogonium. Witschi ('14) states that the number of cells formed from the primary oogonium in *Rana* is at least thirty-two in some cases, but often fewer, while in the testis the number is greater. In the turtle *Clemmys*, Munson ('04) found that the number of divisions was three, each cell thus giving rise to eight cells.

It is impossible to distinguish oogonia and spermatogonia in the lamprey until the larva is about 35 mm. long. Before that time the cells in all larvae appear structurally alike and divide in a similar manner. The centrosphere with its centrosome corresponds to the yolk nucleus described by Lubosch ('04) in the larvae of lampreys about 4 cm. long. He describes it as an oval, clearly defined body of the same structure as the surrounding plasma, but not staining so deeply. Surrounding it, like a membrane, he found deeply staining granules. Lubosch believes that the yolk nucleus introduces the process of yolk-building; but this cannot be its function in very early stages (larvae up to 35 mm. in length) while the cells are still dividing and long before there is any formation of yolk. In these stages it probably functions in cell division in the germ cells as it does in other cells. During the subsequent growth period it may play some part in the process of yolk-building.

The vitelline body is probably present in all indifferent germ cells. It seems to be a permanent element in the germ cells of the lamprey, but it was not possible to determine its origin. King ('08) believes that in *Bufo* it must be considered as a secretion product of the cytoplasm itself, but she thinks it not improbable that a fluid, possibly an enzyme, may pass from the nucleus into the cytoplasm and there cause the formation of the body, and that this enzyme, while in the nucleus, may be in the form of plasmosomes. Dodds ('10) has described a body in the cytoplasm of the early germ cells of *Lophius* and believes that it is a mass of plasmosome material that has been separated and

cast out of the nucleus. The act of extrusion was not observed. The body found by Dodds, however, has only a transitory existence in the cytoplasm, so it cannot correspond to the vitelline body. Since the vitelline body is found to be a very prominent structure in the oocyte of the lamprey, a full discussion of it will be reserved for a future paper which will describe the growth period of the egg.

Witschi ('04) found that sex could be distinguished in frog larvae before there was any differentiation of the germ cells, by the fact that in the larvae destined to become females the germ cells remained along the periphery of the gland, while in those destined to become males the cells migrated into the interior of the gland in very early stages. No differences of this kind have been found in the larvae of the lamprey during the indifferent period. Neither are any genital cavities formed in the gland by means of which the sexes may be distinguished previous to germ-cell differentiation, as is the case in amphibians.

3. *Summary of the period of secondary division.* During the period of secondary division (larvae 20 mm. to 35 mm. long) the germ cells multiply by frequent mitoses. The resulting cells may remain together after division, enclosed by a common follicle, or they may become separated by the migration of follicle or peritoneal cells between them. The result is that in all glands both cysts and isolated germ cells are found. The germ cells are distinguished from the somatic elements of the gland by their size, the structure of their cytoplasm, and the form and structure of their nuclei. There is no indication that germ cells are derived from somatic cells or somatic from germ cells. Usually most of the cells of a cyst are found in one or another phase of mitosis at the same time, but some of the cells of the cyst may be in a resting stage while others are dividing. In the cytoplasm of the germ cells a centrosphere with a centrosome is often visible; also a vitelline body of unknown function and origin. Spermatogonia and oogonia cannot be distinguished during this period. Neither are there any other characters by means of which future males and females may be distinguished.

*D. Period of sex differentiation*

1. *General statement.* The period following that of secondary multiplication of the germ cells is characterized in dioecious animals by the differentiation of male and female individuals. The latter may be distinguished from the former by the appearance in the germ gland of yolk-filled oocytes. This phenomenon is accompanied or preceded by somatic changes in the germ gland and elsewhere. The somatic differentiation may take the form of changes in the structure of the germ gland, the appearance of accessory reproductive organs peculiar to one sex or the other, or the development of other secondary sexual characters.

In the lamprey there are no secondary sex characters developed until after metamorphosis, before which the sexes cannot be distinguished except by an examination of the germ gland, and in early stages even the germ gland does not form a criterion of the future sex of the animal.

2. *Sex characters in the adult brook lamprey.* The reproductive gland in the adult lamprey is unpaired and is suspended by a mesentery from the middorsal line. Previous to spawning, it fills practically the whole body cavity in both sexes. The surfaces of both the male and female glands are thrown into more or less oblique folds, more easily seen in the testis than in the ovary. The testis is made up of numerous cysts filled with spermatozoa and enclosed by follicle cells. It is supported by the mesorchium, from which connective tissue cords radiate into the body of the gland. In the ovary each ovum is enclosed by follicle cells as are the cysts in the testis. Connective-tissue cords, similar to those of the testis, radiate into the body of the gland from the mesovarium above. The number of ova varies in different females as also does their size. In the same animal, however, the ova do not vary greatly in size.

The cysts in the testis and the ova in the ovary may be considered homologous structures. In the one case the germ cells have continued to divide, while in the other case they have stopped dividing early in the life of the animal and have entered upon a period of growth. The greatest amount of growth takes place in the female after metamorphosis, while in the male

metamorphosis is followed by a period of very rapid multiplication of the germ cells. In both sexes the germ cells reach the outside through abdominal pores. These open in each sex into a common urogenital sinus which terminates externally in a urogenital papilla.

There are only a few external sex-distinguishing characters. The urogenital papilla is short and wide in the female, with a large opening at the end, while in the male it is long and slender with a small opening at the tip (fig. 66). Both sexes have a lateral fold of skin on each side of the papilla. In the female the papilla is hidden by these folds, but in the male it extends beyond the folds as a prominent structure. In the female a small anal fin, connected with the caudal fin by a low ridge of skin, lies directly behind the urogenital papilla, while in the male the anal fin is absent or rudimentary. Loman ('12) states that in the European brook lamprey the anterior dorsal fin is always lower in the female than in the male, and Gage ('93) found the cranial end of the second dorsal fin always swollen in the female of the American brook lamprey.

From the above description it will be seen that the reproductive organs in the lamprey are reduced to extreme simplicity. The sex glands and the accessory structures, by means of which the reproductive elements are extruded, are morphologically similar in the two sexes. This point is emphasized because of its bearing on the tendency of the animal toward hermaphroditism.

3. *Changes in the germ gland during the period of sex differentiation.* In larvae less than 35 mm. in length the majority of the germ glands are in an indifferent condition as regards sex. During these stages the germ cells are scattered irregularly through the germ gland, either singly or in cysts; either in a resting condition or in various phases of mitosis, but they are all alike. This indifferent period is followed by one in which distinct sex characters appear in the germ glands through the development of oocytes found both in the synaptic and growth phases (tables 1 and 2). Whether the germ glands eventually become ovaries or testes, they all develop this female character, and the animals may therefore during this stage be considered intermediate as to sex or hermaphroditic.

During this period (larvae 35 mm. to 70 mm. in length), therefore, one may find in any germ gland, germ cells and cysts which are in all respects like those of the indifferent period, as well as germ cells, located singly or in cysts, which are in the various stages of the synaptic phase or which have entered the growth period.

To avoid confusing the germ cells which are in the various phases of mitosis with the cells that had already entered the prophase of meiosis, it seemed necessary to follow as carefully as possible the various steps in the two processes. On account of the small size of the cells and the large number of chromosomes present, it was very difficult to get a complete history, but the main features were worked out.

*a.* Changes during mitotic division. The resting germ cell. The resting cell (fig. 41) has a spherical nucleus in which the chromatin material is in small and large granules or masses, united by fine achromatic threads. Two rather large plasmosomes, at some distance from each other, are present in the nucleus. In cells stained in iron haematoxylin and afterward destained so long that the chromatin masses can no longer be seen, the plasmosomes retain the stain and stand out as two very distinct elements. The formed elements of the nucleus are surrounded by a clear homogeneous nuclear sap, and the whole nucleus is enclosed by a nuclear membrane. The amount of cytoplasm is rather small. An astrosphere with a centrosome lies along one side of the nucleus, although the centrosome is not always visible. A vitelline body sometimes occurs. The cytoplasm is granular and, in cells, fixed in Meves' solution and afterward stained in iron haematoxylin, mitochondria, in addition to finer protoplasmic granules occur.

Prophase. During the first phases of mitosis (fig. 42), the amount of chromatin increases greatly. The original chromatin granules of the resting cell grow in size but remain united by linin threads, so that the whole gives the appearance of a network with conspicuous masses of chromatin at the crossings of the threads. The two nucleoli remain distinct as before.



A little later (fig. 43) the chromatin masses become still more conspicuous, but they continue to be held together by the achromatic threads. The nucleoli are still large, but generally one is considerably smaller than the other. They are closer together than in the preceding stage.

The chromatin granules or masses soon become separated from one another by the breaking of the achromatic threads which up to this time have been holding them together (fig. 44). Each mass is now clearly a short globular chromosome. It was impossible, however, to count the chromosomes with any degree of accuracy, either at this or any other stage. At this time only one nucleolus is visible. The nuclear membrane is still intact. There is some indication that the chromosomes are arranged in pairs.

In the next stage (fig. 45) the nuclear membrane disappears and the chromosomes come together in a mass at about the equator of the cell. The two centrosomes occur at opposite poles, and achromatic spindle fibers extend from the centrosomes to the chromatin mass. The nucleolus is no longer visible and its fate is not known. A polar view of this stage is shown in figure 46. The chromosomes are so closely massed that it is difficult to distinguish each individual chromosome.

Metaphase. Although numerous dividing cells were examined, none was found in which the splitting of the chromosomes could be observed. It seems likely that this process takes place so rapidly that the chances of finding a cell in this stage are slight. Furthermore, the chromosomes are so closely massed together on the equatorial plate that their division would be difficult to observe.

Anaphase. Figures 47 and 48 represent cells in early anaphase. The daughter chromosomes have already begun their migration to the opposite poles. Occasionally bodies having the appearance of chromosomes are found outside of the spindle in the cytoplasm of the cell, or they may be scattered along the spindle threads.

Telophase. In this stage (figs. 49 and 50) the daughter chromosomes have separated from each other and form irregular

groups at opposite poles. The spindle is still distinct. The cell is constricted in the middle and the two daughter cells are about to separate. Sometimes a deeply staining strand or thread remains between the two chromatin masses after they are some distance apart (fig. 49). In a slightly later stage a distinct mid-body is seen along the line of separation (fig. 50). Soon after this the two daughter cells separate, a new nuclear membrane forms around each daughter nucleus, and the two nuclei are in a stage of reconstruction. The two daughter cells are smaller than the mother cell (fig. 51). Small chromatin masses reappear and are united by connecting achromatic threads. At least one nucleolus occurs in each cell. After division the cells enter a period of growth until they have assumed the structure and size of the mother cell.

Discussion. There is nothing strikingly peculiar in common mitosis of the germ cells of the lamprey and only a few observations need further comment. First, the process of division is probably not very rapid. This statement is based on the fact that the number of germ cells does not at any time increase very fast. From the number of germ cells in the mitotic phase at any one time, it is evident also that the karyokinetic period must be rather long, probably occupying several days.

The chromosomes are more or less rounded and stand out most clearly in the middle prophase (fig. 44). The number of chromosomes is very large, and they often give the impression of being in pairs. This may mean one of two things: either that the chromosomes have already divided during the prophase stage before they have reached the equatorial plate or that the maternal and paternal chromosomes remain associated during the prereduction stages. The latter was found by Chubb ('06) to be the case during the multiplication period in the germ cells of *Antedon*. Stevens ('07, '08) found that in *Diptera* a pairing of the chromosomes took place in germ cells far removed from the reduction stages and occurred in connection with each oogonial and spermatogonial division. Metz ('16) has reinvestigated the problem in *Diptera*, and from a study of about eighty species has come to the conclusion that in somatic, as well as in germ

cells, the chromosomes are associated in pairs. The paired condition persists throughout the various phases of cell division. An association of maternal and paternal chromosomes is apparently effected during early cleavages, and probably before the first cleavage, thus continuing from the fertilized egg to the adult stage. In *Diptera* the pairing is side by side (parasyn-desis) and similar to synaptic pairing. The pairing, according to Metz, comes about through a physicochemical similarity of the homologous maternal and paternal chromosomes.

In the lamprey the granules on the achromatic network of the resting nucleus form the centers for the reconstruction of the chromosomes. There is little doubt that these granules actually represent chromosomes in all the various phases through which the cells pass; for this reason one may speak of a visible continuity of chromosomes from one cell generation to the next. Thus the individual chromosomes in the germ cells of the lamprey never lose their identity during mitosis.

The origin, function, and fate of the plasmosomes remain obscure in the cells of the lamprey. In the resting cell there are usually two plasmosomes which appear approximately of the same size and which lie some distance apart. As the phenomena of prophase advance, they approach each other, and somewhat later only one is present. Whether a fusion of the two takes place or one dissolves and disappears at this time is not known. During late prophase the single plasmosome also disappears or at least is no longer distinguishable among the chromosomes. During the telophase a new plasmosome soon appears in each daughter cell and, as the growth of the cell progresses, a second plasmosome also appears. It is likely that the plasmosomes dissolve during cell division to be formed *de novo* in the daughter cells.

b. Synapsis phase of the oocytes. After an indefinite number of divisions, some of the primordial germ cells (oogonia), which lie singly or in cysts, undergo a series of changes preliminary to the stage of actual growth. When the cells have entered this stage they are termed oocytes of the first order (table 1).

Very little was known concerning the processes that take place in the oocyte of any animal preliminary to its growth period, until von Winiwarter ('01) published his extensive observations of this period in the cat and in man. Based upon changes which take place in the nucleus, he divided the transition period from the oogonium to the oocyte into the following periods: 1) Noyaux protobroques; 2) noyaux deutobroques; 3) noyaux leptotènes; 4) noyaux synaptènes; 5) noyaux pachytènes; 6) noyaux diplotènes, and, 7) noyaux diptyes. In a later publication by von Winiwarter and Sainmont ('09) another stage has been added between the first two, namely, noyaux poussieroides. Other investigators have divided the period differently and have applied other terms to the different phases, but on the whole the processes taking place in all animals in which the synapsis phase has been studied seem to follow the general course outlined by von Winiwarter. In the lamprey the changes correspond in the main with those of other forms studied. An abbreviated list of the above stages has already been given (table 1).

Nuclear changes. Early leptotene. After the last oogonial division the germ cell enters a period of rest, during which the chromatin of the nucleus becomes broken up into small particles (fig. 52). These are scattered throughout the whole substance of the nucleus so as to make its contents appear almost homogeneous. There are no very distinct chromatin bodies in the nucleus at this stage and the only stainable parts are a very fine network and two very distinct plasmosomes. This stage corresponds to the stage in the germ cells of the cat described by von Winiwarter and Sainmont ('09) as 'noyaux poussieroides,' and to the stage in the germ-cell history in *Proteus* described by Jörgensen ('10) as 'erste Zerstäubung.'

Sonnenbrodt ('08) studying this period in the germ cells of the chick, found that the chromatin at this stage was very small in amount, and he believes that the period succeeding the last oogonial division is devoted largely to the formation of new chromatin. He says it "besteht in der Hauptsache in der chromatin Aufnahme oder richtiger Chromatinbildung." In the lamprey, too, the chromatin network begins to reappear at a somewhat

more advanced stage, but for a long time the chromatin remains in a finely divided condition. The nucleus appears to grow larger as the period progresses. The two nucleoli persist throughout the period.

Bouin ('01) studied this period in the oocyte of the frog, and called the cells of this stage 'ovogonies de transition.' He described the nucleus as losing its membrane at this time so that there was a free communication between the nuclear and cytoplasmic substances. This has not been confirmed for other forms and it is certainly not the case in the lamprey.

Late leptotene stage. During this stage (figs. 53 and 54) the chromatin network becomes much more distinct. The whole nucleus is now filled with chromatin threads which cross one another in various ways. Irregular thickenings are found on the chromatin threads not only at their intersections, but in other parts as well.

Synaptene stage. During this stage (fig. 55) the chromatin becomes massed together along one side of the nucleus in the form of an irregular tangle of rather thick, deeply staining threads, and forms what has been termed a 'contraction figure.' This is the stage described by von Winiwarter as the synaptene stage and by Maréchal ('04) as the 'bouquet stage.' On the side of the nucleus on which the chromatin is concentrated, the individual threads can no longer be distinguished; but in the clearer parts of the nucleus many of the ends of the chromatin threads extend out from the concentrated mass, sometimes as far as the nuclear membrane of the opposite side. Occasionally a nucleolus, which later appears to be lost, occurs during the early phases of this stage. It is possible that later, when it is not visible, it is hidden among the chromatin threads of the contraction figure. If in reality this be the case, it indicates a tendency of the chromatin to concentrate around the nucleolus during this stage; for otherwise, if the nucleolus be present, it should be found occasionally in the clearer portions of the nucleus. Some investigators have termed this period the synapsis stage, because in many forms the chromatin threads come together in pairs at this time. McClung ('05) has called it the 'synizesis stage,' and this is a more appro-

priate term, since apparently synapsis does not always occur during this period. In the case of the lamprey it was not possible to find any pairing of the chromatin threads at this time, although hundreds of cells were examined.

Pachytene stage. In the pachytene stage (fig. 56), the chromatin material again becomes uniformly distributed throughout the nucleus. It is now in the form of thick threads which appear more or less continuous in some places, but are generally broken up into segments. One large nucleolus appears. There is no indication that the chromatin threads are paired.

Diplotene-dictyate stage (diakinese) (figs. 57, 58, 59, 60, and 62). In an oocyte somewhat farther advanced than the above, the whole chromatin network has become broken up into definite chromosomes, and the paired structure of every chromosome is very apparent, a condition which persists throughout the early part of the growth period and probably up to the time of maturation. It is very difficult, however, to follow the history of the chromosomes during the later growth period, since the nucleus becomes very large and the chromatin material may be scattered throughout its whole extent. The nucleolus at this time is large and almost spherical. It has not been possible to observe any relation between the nucleolus and the chromosomes at this period of development. Many of the paired chromosomes lie against the nuclear membrane, but they may occur also in various other parts of the nucleus. Very often they are arranged in the form of tetrads which are best seen along the nuclear membrane (fig. 61). There appears to be no regularity in the arrangement of the chromosomes. The dictyate stage (diakinese) continues during the growth period of the oocyte.

Cytoplasmic changes. The nuclei of the oogonia are surrounded by a small amount of granular cytoplasm. Often no visible cell boundaries are present, although favorable sections show that the cells do not form a syncytium, but are morphologically independent of one another, in spite of the fact that no true cell membrane is found. In cells, fixed in Meves' solution or in other solutions that fix mitochondria, these occur in great numbers. They are usually granular and appear to be more

or less grouped. There is no evidence that they are derived from the nucleus, so they may be considered as true cytoplasmic bodies. The mitochondria in the early oocytes of the lamprey are not essentially different from those found in the indifferent germ cells (figs. 41 and 52).

During the progress of the early nuclear changes, up to the time of the synaptene stage, there is a gradual decrease in the amount of cytoplasm and a gradual disappearance of the mitochondria. After the synaptene stage the amount of cytoplasm increases again, but no study was made of the mitochondria subsequent to the synapsis phases.

In the undifferentiated germ cells there is an astrosphere near the nucleus, even in the resting cells. Sometimes a minute centrosome has been distinguished in the middle of the astrosphere. The astrosphere may be distinguished from the surrounding cytoplasm by its more granular appearance. During the middle synapsis phases it seems to disappear with the decrease in the amount of cytoplasm, but it reappears in the early growth period and remains through this whole period as a very prominent structure (fig. 60).

The vitelline body may be traced through the various phases of the synaptic period, and during the growth period it becomes a very conspicuous structure (figs. 60, 62).

Discussion. The general history of the period has been outlined above, but certain features require further discussion. The changes taking place in the cells during this period initiate the period of heterotypic or reduction division. This is a period through which all germ cells apparently must pass before they can become functional ova or spermatozoa. In the female cell these changes take place at a very early stage in the development of the animal, and in the case of the lamprey they precede the maturation period proper by at least two or three years. In the male lamprey these changes occur much later in the life of the individual and usually precede the maturation divisions by only a very short time, probably not more than three or four months.

I have made a study of the synaptic phases of the male germ cells in the lamprey and found the process of development to be

much like those of the female. In both sexes the germ cells presumably come out of the synaptic phase with the number of chromosomes reduced to one-half the number found in the pre-synaptic cells. The subsequent history of the cells, however, differs in the two sexes. In the female the cells grow to an enormous size by the accumulation of yolk, while in the male there is very little growth.

Aside from the apparent pairing of the chromosomes during the period of multiplication, no other instance of chromosome pairing was observed in the cells before they have reached the diplotene stage; but it cannot be said with certainty that a doubling does not take place before this stage during the synaptic phase. In some forms which have been investigated, synapsis seems to take place during the synaptene stage, or during the period of transition from the leptotene to the synaptene. Von Winiwarter ('01) figures a pairing of the chromatin threads in the germ cells of the rabbit during the early synaptene. Von Winiwarter and Sainmont ('09) describe a similar condition in the cat. In *Proteus* also, according to Jörgensen ('10) the leptotene stage is followed by a stage which shows a double nature of the chromatin threads. In *Bufo*, King ('08) figures double chromatin threads for the first time after the synaptene stage. Maréchal ('04) observed the double structure of the threads during the synaptene stage in *Pristiurus* and *Scyllium* and later ('07), in *Ciona* and *Amphioxus*. Janssens ('04) found that in *Triton* the reduced number of chromosomes, or chromatin filaments, appears shortly after synizesis and that these filaments subsequently split longitudinally forming two sister threads which remain together. d'Hollander ('04) found a massing of chromatin (synizesis) in the oocyte of the hen before synapsis.

The phenomenon of synizesis (McClung, '05) has been found by various investigators to occur in the oocyte of invertebrates as well as in vertebrates; it seems to be a universal phenomenon of the early heterotypic prophase. Chubb ('06) thinks that synapsis takes place in *Antedon* during the oogonial divisions, and that it is followed by still one more division. In *Sycon* Jörgensen ('10) thinks that the reduced number is present in



the oogonia. According to these investigators, synapsis may take place previous to synizesis, and the two phenomena probably have nothing in common. Most investigators, however, agree that the double nature of the chromosomes is first visible during a late stage of the heterotypic prophases, but their interpretations of the doubling vary. Some consider it a suppressed mitosis (Hertwig, '08; Matscheck, '10, and others), while the majority of workers on germ cells look upon it as a pairing of parental chromosomes similar to that which takes place in the male germ cells previous to the maturation division. Very little light can be thrown upon this subject by the lamprey, since it was found impossible to count the chromosomes before or after synapsis. To all appearances, however, the chromosomes enter synizesis in the univalent condition. The bivalent nature of the chromosomes is not observable before the diplotene stage.

The meaning of the 'contraction figure' has been variously interpreted. Some investigators consider it simply an artifact due to poor preservation (Janssens, '05; Jörgensen, '10, and others). Maréchal and Saedeler ('10) insist that it is not an artifact in *Raja clavata*. King ('08) has shown that it is a perfectly normal condition in the toad. In the lamprey it appears to be a normal phenomenon, and forms a stage in the morphological changes which take place in the oocyte at this time. In the same gland were cells in the contraction phases, other cells in the various stages of the synapsis phase, normal resting cells, and cells in the different phases of mitosis. Degenerating cells also occurred in most glands, but no evidence was found to indicate that the contraction figure is a phase in the process of degeneration. Even in the same cyst, there are contraction figures side by side with resting cells and cells in other stages of the synapsis phase. It must be concluded, therefore, that the phenomena connected with synizesis in the lamprey are perfectly normal and due to some peculiar condition of the cells at this time—a condition the nature of which is not yet understood.

Whether the contraction figure is normally formed around the nucleolus or on the side next to the centrosome could not be determined. A body sometimes occurs in the cytoplasm near the

nucleus on the side toward which the contraction figure is formed, but this was taken to be the vitelline body since it may often be found in other parts of the cells. No distinct centrosome or attraction sphere could be found in the cells at this time. Jørgensen ('10) figures a very distinct astrosphere during the bouquet stage in *Proteus*, but he was not certain of the presence of a centrosome. He also found that during this period there was an extrusion of chromatin material from the nucleus into the cytoplasm on the side next to the astrosphere. This was not found to be the case in the lamprey, in which the nuclear membrane appears to be intact throughout the period of transformation of the germ cells into growing oocytes. No centrosome was found by King ('08) in the germ cells of the toad during the synapsis period, and she concludes that probably the egg centrosome disappears after the last oogonial division. Lams ('07), on the other hand, observed a centrosome in the germ cells of the frog during the bouquet stage.

Although no centrosomes or astrospheres were found during the synapsis phase in the oocytes of the lamprey, they are not permanently lost, for they reappear somewhat later in the growing oocyte. It is probable that special technique might make them visible also during the synapsis stages. Jørgensen holds that the centrosome is functional in connection with the convergence of the chromosomes along one side of the nucleus during the bouquet stage, with the radiations in the cytoplasm through the orientation of plasma inclusions, and with the formation of a permeable region in the nuclear membrane where chromatin bodies may be extruded from the nucleus. In the lamprey no extrusion of visible chromatin material from the nucleus at this stage has been observed, but there is ample evidence that such extrusion takes place in the dietyate stage, although there appears to be no special area of the nuclear membrane over which it occurs.

It has been shown that two nucleoli are present in the germ cells of the lamprey during the multiplication period. During mitosis these are reduced to one, which also later disappears. In the resting cells after mitosis a single nucleolus appears and

shortly afterward a second. When the cells enter the synapsis phase, the nucleoli again disappear, apparently during synizesis. When the cells enter the growth phase, only one nucleolus appears in each cell. This remains during the whole period as a very prominent spherical structure. Whatever may be the function of the nucleoli, they are, as shown by their reaction to stains, true plasmosomes, and not composed of chromatin material. This is contrary to the idea of Lubosch ('03), who believes that in the lamprey and in other forms the chromatin material is stored in the nucleolus during the growth period of the egg. This view is based largely on the fact that the chromosomes seemingly disappear during the later stages of growth. Lubosch also thinks he has evidence that the maturation chromosomes are derived from the nucleolus. I have found some evidence which indicates that the maturation chromosomes appear in the clearer portions of the nucleus and do not come from the nucleolus.

Von Winiwarter and Sainmont ('09) found in the nucleus of the oogonia of the cat, at the time when the cells were preparing for mitosis, an elongated body which stained like chromatin. Often it had a horseshoe shape, and it was larger than the other bodies of the nucleus. During mitosis it divided, but much more slowly than the chromosomes. In the oocyte it was often attached to the plasmosome, but sometimes it was free. When dividing, it split longitudinally, and during the growth period it disappeared. The body was supposed by the authors to be a sex chromosome (monosome). Guthertz ('12) found a similar body in the spermatocytes of the cat, but came to the conclusion, on account of its staining reaction, that it was a true plasmosome "*der einen Gestalt ein Heterochromosome in Herteropyknose vertauscht.*" Guthertz doubts that the body observed by von Winiwarter and Sainmont was a true sex chromosome, since no differential stain was used by them. Furthermore, there should be two sex chromosomes present in the oocyte of the cat, if such bodies are present at all, since in this form the male is apparently heterozygotic with respect to sex.

There is considerable danger of misinterpreting nuclear bodies. Many of the structures described in the germ cells of vertebrates

as sex chromosomes may be plasmosomes and have nothing to do with the determination of sex. Wilson ('13) found a body in the spermatocytes of *Pentatoma* that simulated an accessory chromosome, and which he called a 'chromatoid body.' A similar body has been found by Wodsedalek ('14) in the spermatocytes of the horse and by Bachhuber ('16) in the spermatocytes of the rabbit. More recently, Swingle ('17) describes what he considers to be the same kind of body in the spermatogonia of the frog (*Rana pipiens* and *R. catesbiana*). The body was in all these cases of cytoplasmic origin and was found with the chromosomes only during mitotic division.

An examination of the various figures given of a so-called sex chromosome in vertebrates reveals a striking resemblance to plasmosomes similar to those that are found in the early oocyte of the lamprey. Stevens ('11) describes such bodies in the spermatocytes of the guinea-pig; two such bodies were found by Wodsedalek in the spermatocyte of the pig; similar bodies were found by Levy ('15) in the spermatocyte of the frog. Guyer ('09, '16) has described such bodies in the spermatocytes, in the oocytes, as well as in the body cells of the fowl, and ascribes to them a sex-determining function. Finally, Jordan ('14) has found such a body in the germ cells of various mammals. Bohring and Pearl ('14) have studied the body found by Guyer in the domestic fowl, and have come to the conclusion that it is not a sex chromosome.

At the present time the status of the sex chromosome in the germ cells of vertebrates is very uncertain. It is unfortunate that the subject has been studied almost exclusively in the male germ cells. Von Winiwarter's account of such a body in the oocyte of the cat and Guyer's description of the body in the female germ cells of the fowl seem to be about the only accounts dealing with the sex chromosomes in the female germ cells of vertebrates.

It is generally assumed that when the spermatozoa in a species are dimorphic, the female of the same species produces eggs only of one kind. These correspond in their chromosome make-up to the male cell possessing the sex chromosome. All the eggs, therefore, in such species possess accessory chromosomes. On the

other hand, it is assumed that if the female produces two kinds of eggs which differ in their chromosomal structure by the presence or absence of a sex chromosome or chromosome complex, the male must produce only one kind of spermatozoa. In this case one-half of the ova should correspond to the spermatozoa in their number of chromosomes. Guyer ('16) says that in the fowl, where it has been shown experimentally that the female is heterozygous for sex, there are also two kinds of spermatozoa. He believes it is probable that only the spermatozoa containing the odd element become functional.

In the lamprey no evidence has been found of the presence of an accessory chromosome in the oocyte during the synaptic phase, the growth period, or the maturation division stage. A search has also been made for this body in the spermatocytes during the various stages of maturation, but without success. If, as seems to be the case in some invertebrates and in *Necturus* among vertebrates (King, '12), the sex chromosomes might be united with other chromosomes, it would be extremely difficult to find it in forms like the lamprey where the chromosomes are very small and numerous. Observations on the lamprey can neither affirm nor deny the existence of sex chromosomes which might be responsible for sex. It can only be said that such a body has not been found. Whether or not the assumption of the presence of such a body is necessary to account for sex in forms like the lamprey where the sex potentialities are so equally balanced, is a question which will be discussed later.

I have not found in the lamprey a transfer of visible material between the nucleus and the cytoplasm of the oocytes during the synaptic period, but have found that there is an intimate relation between the two parts of the cells. The absolute amount of cytoplasm decreases greatly during this stage, and it is not until the nucleus enters the diplotene phase that the cytoplasm begins to grow again. All the energy of the cell seems to be devoted to nuclear changes in the early oocyte and to cytoplasmic changes during the growth period which follows synapsis. The mitochondria, which are abundant in the oogonia, disappear during the synaptic phases or, at least, can no longer be seen. In

this respect they seem to behave like zymogen granules in gland cells. In the resting gland cells the zymogen granules are very abundant, but in a cell which is secreting they decrease in number and size, and may disappear entirely if activity continue.

Most of the mitochondria in the germ cells of the lamprey are spherical, but occasionally rod-shaped ones may also occur. The theory that the early germ cells may be distinguished from the somatic cells by the shape of the mitochondria has been developed by Rubaschkin ('10) for mammals, Tschaschkin ('10) for birds, and Aunap ('13) for fishes. These investigators think that the mitochondria of the germ cells are spherical, and that during the process of development of the embryo they become chain-like and finally rod-shaped in the differentiated tissue cells. The primitive character of the germ cells is, therefore, according to these investigators, indicated by the fact that they possess granular mitochondria after the other cells of the embryo show rod-shaped ones. That this is a universal character of the early germ cells has been denied by von Berenberg-Gossler ('12) and others. Von Berenberg-Gossler found that in the individual germ cells of the duck and the chick, the shape of the mitochondria may vary from granular and chain-shaped to rod-shaped. Firket ('14) also found that in the germ cells of the chick, the shape of the mitochondria is not constant.

In the oocyte of the lamprey the mitochondria may be found again after the beginning of the growth period. They are cytoplasmic structures and not related to the chromidia which are so abundant in the growing oocytes of the various stages. This is in agreement with Schaxel ('10, '11) and others who consider the chromidia to be of nuclear origin and the mitochondria to be of cytoplasmic derivation.

Meves ('08), the first to study the mitochondria in embryonic cells, upheld the theory which had previously been advanced by Benda ('03) and others, that the mitochondria are bearers of cytoplasmic heredity. This theory has since been advocated by Duesberg ('08, '10) and others. Those who adhere strictly to the chromosome theory of inheritance are opposed to it. According to Cowdry ('16), the chemical nature of the mitochondria

seems to oppose the idea that they are individual constant bodies in the same sense that the chromosomes are considered to be so. It is more likely that they play an active rôle in the metabolic activities of the cell and that they may vary in number, size, and shape as the activity of the cell varies.

c. History and fate of the germ cells during the period of sex differentiation. This period (table 2) includes larvae from about 35 mm. to about 70 mm. long. Figure 68 is a cross-section through the germ gland of a larva 54 mm. long, from the middle of this period. It shows many large, growing oocytes, as well as many cysts. A comparison of sections from various larvae shows that the oocytes are formed from germ cells which have entered synapsis, either while isolated or while constituent elements of small cysts, usually of less than eight cells. In the latter case the cysts become broken up by the penetration into them of follicular cells, so that each of their contained cells, while still in the synaptic phase or in the early growth phase, becomes isolated within its own follicle. Thus, as shown in figure 68, nearly all of the growing oocytes are sooner or later isolated cells. In addition to these cells the section contains numerous large cysts, and of these there are two kinds. In one kind the germ cells are still indifferent, as shown by the fact that they have not entered synapsis, but are dividing by typical mitosis. Such a cyst containing two cells in mitosis is shown at the left of the figure. In the second kind of large cyst the cells have entered synapsis. Such cysts are shown to the right of the figure and are recognizable by the different behavior of the chromatin. In large cysts the cells which enter synapsis rarely become growing oocytes, but sooner or later degenerate, until finally they and their enclosing follicles break up and disappear. A longitudinal section of a gland from a larva 59 mm. long, in which two such degenerating cysts are found, is shown in figure 69. The cyst at *deg.cy.2* is in a later stage of degeneration than the one at *deg.cy.1*. A detailed drawing of such a cyst from another section is given in figure 65. The cells of such a cyst rarely get beyond the synize-sis stage before degeneration sets in. Degeneration begins with a condensation of the chromatin into solid masses, as shown at

*deg.g.* in the figure. The cytoplasm disintegrates and finally the chromatin masses break up and the fragments are scattered through the whole cyst. Such fragments are shown at *g.f.* Other stages occur showing the various steps up to the final dissolution of the cysts. No large cysts containing cells in synapsis have been found to break up by the inward migration of follicle cells. The final fate of all of them and of their enclosed cells is degeneration. Rarely, however, a single cell of such a cyst may enter the growth phase. A cyst containing such a cell is shown in figure 64. This contains a large oocyte among the smaller cells of the cyst. Whether such oocytes continue to grow and form functional ova is not known, but it is certain that nearly all of the growing oocytes are derived from isolated germ cells or from the breaking up of small cysts. Other sections, showing both cysts and oocytes in about equal numbers, are given in figures 70 and 71 from larvae 55 mm. and 62 mm. long, respectively.

In the middle of the period of sex differentiation, therefore, there are in the same gland undifferentiated germ cells that are still dividing; germ cells in the various prophases of heterotypic division; cells that have entered the period of growth, forming recognizable oocytes, and cells undergoing degenerative changes.

The gland described above represents only one type of germ gland during this period—a type in which the number of growing oocytes and cysts is approximately the same. There are other glands with very few growing oocytes, and still others in which there are very few cysts. Figure 72 is a section through the germ gland of a larva 50 mm. long in which there are very few growing oocytes, and none of these are shown in the section. The gland is filled with cysts, some of which contain undifferentiated germ cells, while some contain cells in the synaptic phases. It is often found that if a gland seems free from growing oocytes when examined superficially, more careful search usually reveals at least a few of them scattered about through the gland. In one such gland only one growing oocyte could be found.

Figure 73 is a section of the germ gland of a larva 71 mm. long. In this larva there are only a few growing oocytes, only one of



which is shown in the section. Numerous cysts are present, some with cells in synapsis, others with degenerating cells, and still others with undifferentiated cells.

Figure 74 shows a cross-section of a gland from a larve 65 mm. long. In this larva the gland contains practically only growing oocytes, although a few individual cysts are scattered throughout the gland. Such cysts may contain only actively dividing germ cells or only cells in the various stages of degeneration. Larvae with such glands quite certainly become females. Another gland of this type from a larva 63 mm. long, is shown in figure 75.

Figures 76 and 77 are two sections from larvae 50 mm. and 60 mm. long, respectively. In these the number of oocytes in the gland is greater than the number of cysts.

Germ cells of the various types mentioned above are distributed throughout the germ glands of this period, apparently without any regularity and without any relation to one another or to the somatic parts of the gland. All of the types may occur along the periphery of the gland or in the deeper portions. Cells may enter the synaptic phases whether they lie singly or in cysts, and irrespective of the position they occupy in the gland. Furthermore, no difference has been observed between the follicular cells surrounding those germ cells which have entered the synaptic phases and those enclosing the resting and dividing cells. There is, then, no indication that the somatic environment has anything to do with the initiation of the synaptic phases. The fact, however, that the cells of large cysts which have entered the synaptic phase usually degenerate indicates that the environment of the cells at this time may determine whether or not they shall form growing oocytes.

In none of the germ glands do all the cells that are destined to form growing oocytes enter the growth phase simultaneously, and therefore, in the same gland one may find oocytes of all sizes, as well as cells in the various stages of synapsis. Figure 78 is a section of the germ gland of a larva 72.5 mm. long, in which are oocytes of various sizes. There is no special limited period during the course of early development when the germ cells show a greater tendency than at other times to change into oocytes.

The change may take place in some cells when the larvae are less than 40 mm. long; while in other larvae no oocytes are found until a much later stage, in some cases not until the larvae are 70 mm. long. Few or many growing oocytes may form in the germ gland in very early stages. In most cases they are formed before the larvae are 70 mm. long, at which stage the sex glands are either predominantly male or predominantly female. A few oocytes may enter the growth phase after the larva is 70 mm. long, especially in glands that are predominantly male. After that time the cysts and undifferentiated germ cells gradually disappear in larvae destined to become females, while in those that become males the oocytes which have reached a considerable size or which may form in the gland subsequent to this time remain in the gland up to the adult stage. One such cell from an adult testis is shown in figure 63. It has been found that in the majority of adult testes such undeveloped oocytes occur.

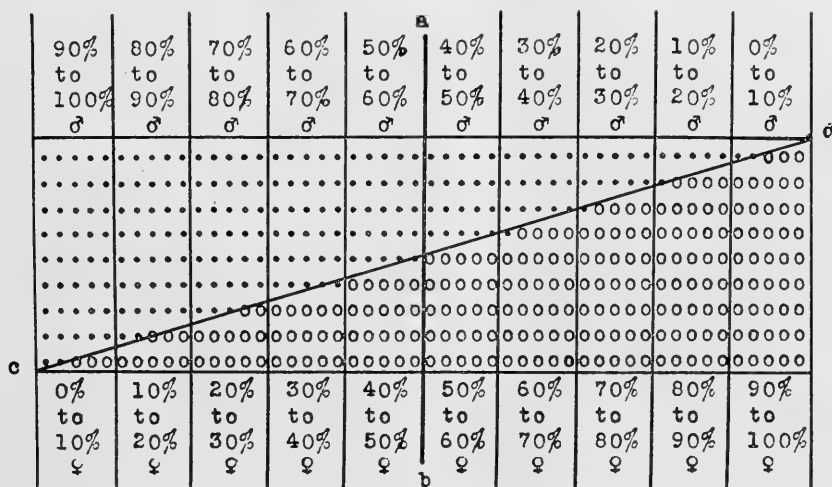
Although the caudal portion of the germ gland remains smaller and less developed than the cranial portion, yet no difference has been found in the structure of the glands in the two regions. The tendency to form oocytes seems to be equally strong in the cranial and caudal portions of the gland.

So, whether the germ glands eventually become ovaries or testes, they all develop oocytes, and this is an undoubted female character. Sometimes only a few oocytes are present, and again, with the exception of a few indifferent germ cells scattered through the gland (singly or in cysts), oocytes may make up the whole of it. Hundreds of glands from young larvae have been examined to ascertain whether or not there are, in addition to the oocytes, any other sex characters which might indicate that the larvae are predetermined to form one or the other kind of sexual individuals, but none have been found. The germ glands vary somewhat in different regions, the result of the presence of blood-vessels and the irregular distribution of the various somatic elements, but the limits of such variation of the somatic parts of one gland are not appreciably different from those of any other gland. The only basis, therefore, for considering a larva male or female is the relative number of cysts and oocytes present in the

gland. If in older larvae of this period, the number of cysts is greater than the number of oocytes, the larva probably becomes a male; if the number of growing oocytes is greater than the number of cysts, it probably becomes a female. In younger larvae of the period this cannot be a reliable criterion, for in some cases oocytes do not apparently begin to form in great numbers until the larvae are over 5 cm. long. Other larvae of the same size show evidence of having formed a large number of

TABLE 4

Diagram showing the relative number of cysts and oocytes in various lamprey larvae during the period of sex differentiation



oocytes in their early stages, but appear later to have formed cysts only. Other larvae show about equal tendencies to form oocytes and cysts during the whole intermediate period.

— The relative number of cysts and oocytes found in the various larvae of this period may be represented in percentages from one to a hundred as shown in the above table (table 4), which should be compared with table 2 (page 12).

The circles in the table represent ova, the small dots cysts. The spaces between the vertical lines may represent individual larvae, and the relative number of cysts and ova in the glands

of each of these is indicated by the dots and open circles above and below the oblique line *cd*. The whole diagram may represent ten larvae of any size between 35 mm. and 70 mm. in length. If a large number of larvae of all sizes between 35 mm. and 70 mm. in length were sorted according to the number of cysts and oocytes present, approximately the same number of them would fall within each of the vertical spaces. Those to the left of the line *ab* would be predominantly male and those to the right predominantly female, as judged by the number of cysts and oocytes present in the germ gland. Those on the extreme left would be more strongly male and those on the extreme right more strongly female than those which would fall in the groups nearer the line *ab*.

Although the future sex of the larvae cannot be determined for a long time after oocytes have appeared in the germ gland, there is apparently developed out of these indeterminate larvae an approximately equal number of males and females. As previously stated, it is very difficult to obtain exact data on the sex ratio in the adult lamprey, since the habits of the two sexes are so different; but it has been found that out of all the adults collected from year to year and at various times of the day as well as at various times during the breeding season, there is only a slight excess of males over females, the ratio being about 118 males to 100 females. Many of the larvae, therefore, which bear the unmistakable female character of possessing oocytes must later develop into males.

*d.* Literature on the period of sex differentiation in the lamprey. W. Müller ('75) found that in larvae of *Petromyzon planeri* 35 mm. long the germ cells were not yet differentiated, while in larvae 50 mm. long oocytes were found. In larvae 65 mm. long he found the ovary and the testis to be fully differentiated. Lubosch ('03) found in the same species that sex differentiation takes place at the end of the first year. The youngest identified ovary was from a larva 40 mm. long.

Lubosch was the first to call attention to the fact that hermaphroditism is of common occurrence in the larvae of lampreys. He examined forty-nine germ glands from larvae of *Petromyzon*

planeri. Among these 10.3 per cent were undifferentiated, 16.3 per cent older undifferentiated (probably young testes), 48.9 per cent true ovaries, and 24.5 per cent mixed glands. He came to the conclusion that the greater part of the hermaphrodite or mixed glands “. . . wird als männliche Anlage gelten können, in der eine kolosale, gleichsam atavistische Anlage von Eiern stattfindet.” He states further: “Es ist anzunehmen, dass diese Eier später während oder nach Metamorphose der Rückbildung anheimfallen werden.”

I published ('14) a summary of some work on the sex glands of the American brook lamprey, *Entosphenus wilderi*. Fifty larvae ranging in length from 20 to 75 mm. were examined for sex. Out of these 46 per cent were regarded as female because the germ glands contained practically nothing but growing oocytes, 10 per cent were taken to be true males on account of the absence of any growing oocytes in the germ gland, and 44 per cent were considered intermediates, for the reason that both cysts and growing oocytes were found in the glands. Since in the adult stage males and females occur in nearly equal numbers and since undeveloped oocytes were found in the adult testes, the conclusion seemed warranted that the intermediates became males.

Two other instances are recorded in which oocytes were found in the mature testis of the lamprey. Beard ('93) found in the testis of a specimen of *Petromyzon planeri* one well-marked oocyte in an individual follicle for every forty sections of  $10\ \mu$  thickness. Ward ('97) describes the occurrence of a single microscopic oocyte in the testis of an adult *Petromyzon*.

Further discussion of juvenile hermaphroditism in lampreys will be found at the close of the next section following the discussion of like conditions in other vertebrates.

*e.* Other cases of juvenile hermaphroditism among vertebrates.

Cyclostomes. Cunningham ('86) found that in all specimens of *Myxine glutinosa* with very immature eggs, the caudal portion of the sex gland had the structure of a testis. In one specimen this testicular portion showed spermatogenesis and a number of spermatozoa. In all the sex glands of specimens with well-

developed ovarian eggs there was, with one exception, no testicular portion. He concluded that in the young state nearly all females were hermaphroditic and that the testicular portion of the sex gland normally disappeared as the eggs became more mature. He believed that fertilization was normally effected by these hermaphrodites, since true males were so rare. Out of hundreds of specimens examined he succeeded in identifying only eight males.

Nansen ('87) also worked on *Myxine*, and came to the conclusion that all the animals are males up to a length of 32 to 33 cm., after which they change sex and become females. He regarded this as a case of protandric hermaphroditism among vertebrates. Dean ('97), as a result of his study of *Bdellostoma stouti*, doubted the conclusion reached by Nansen, as also did Price ('96). It was not, however, until 1904 that Schreiner proved by the examination of hundreds of specimens of *Myxine* that hermaphroditism in this form is of a juvenile character and that each animal matures only one kind of germ cells. Schreiner divided the animals examined into three groups, namely, males, females, and sterile. In the males the testes occupy the posterior portion of the gonads, while the anterior portion may not develop at all or may contain ova arrested in their development and showing signs of degeneration. In the female the ovary occupies the anterior portion of the gonad and is well developed, while the posterior or testicular portion is sterile and only slightly developed. The sterile individuals were of two kinds: those that showed neither follicles nor eggs and those in which both ova and follicles were found. Ova were usually found in the testes of the males, ranging from only one in some specimens to a large number in others. Out of hundreds of specimens examined only nineteen males were found without ova. The evidence obtained by Schreiner and later confirmed by Cole ('05) shows that *Myxine* is not a protandric hermaphrodite, but a juvenile hermaphrodite like the brook lamprey. This same conclusion has been reached by Conel ('17) for *Bdellostoma* as well as for *Myxine*. Conel believes that in the males the sex gland may degenerate with age, and that this accounts for the sterile individuals found by Schreiner.

Teleostei. Certain species of teleosts belonging to the families Sparidae and Serranidae are said to be normally hermaphroditic, and those of the latter family are even said to be self-fertilizing (Brock, '81; Howes, '91). Brock found in certain species of Sparidae that some of the young were hermaphroditic and others unisexual. In the former the ovarian elements did not mature and they became males, while the latter became females. I have shown that in the young lamprey the male part of the predominantly female gonad soon disappears, or is at least in many cases not very conspicuous, so that the impression may be gained by superficial examination of certain individuals that they are pure females. In the male, however, the female character, namely, the presence of oocytes, persists in many cases even up to the adult stage. For this reason one might conclude from the examination of older larvae that the males alone are hermaphroditic or heterozygotic as to sex. This was the conclusion that I first reported ('14). From a more careful examination of earlier stages I have now found that there is essentially no difference between the males and the females with regard to the juvenile hermaphroditic condition and that the apparent purity of the older larval females is due to the fact that the male character in the form of cell nests does not persist for any length of time after the female character has become predominant. Out of the hermaphroditic larvae, therefore, both males and females develop, and not males only as was at first supposed. In bony fishes there are no recent researches on sex differentiation, and the problem of juvenile hermaphroditism in this group needs to be reinvestigated. It may be expected to occur in both sexes.

Amphibia. It has long been known that in toads anterior to the true sex gland there is an organ (Bidder's organ) which has the structure of a rudimentary ovary. In males it persists throughout life, but in females it disappears after the second year in all forms that have been studied with the exception of *Bufo vulgaris*, in which, according to Ognew ('06), it is retained throughout life. Certain cells in this organ are, in structure and development similar to true oocytes. Ognew states that the boundary between Bidder's organ and the germ gland is often quite indefinite, so

that one merges more or less into the other. In many cases oocytes occur even in the center of the testes, and Cerruti ('07) also found follicles which contained spermatozoa in Bidder's organ. With age, however, the boundary between Bidder's organ and the testis becomes more and more definite.

Pflüger ('82), one of the early workers on the sex problem in frogs, came to the conclusion that in recently metamorphosed frogs there are three kinds of individuals, namely, males, females, and hermaphrodites. During development the hermaphrodites become definitive males and females, so that in the adult condition the number of males and females is about equal. Pflüger also found that in certain races of frogs there is a greater tendency toward juvenile hermaphroditism than in others. An examination of recently metamorphosed frogs collected in nature from three different geographical regions gave the following results:

	PER CENT MALES	PER CENT FEMALES	NUMBER EXAMINED
Bonn.....	35.0	65.0	228
Utrecht.....	13.2	86.8	459
Königsberg.....	47.2	52.8	500

Collections of adult frogs from the three regions showed that the number of males and females was approximately equal. The conclusion was reached that the young are often hermaphroditic and do not reveal the final sex condition of the animal.

R. Hertwig ('05, '06, '07) also found that young frogs showed a tendency toward juvenile hermaphroditism. In two laboratory cultures, in which all the larvae were brought beyond metamorphosis, he found forty-three females and eighteen males in one and forty-seven females and eight males in the other. He believed that the females were pure and that only the males were hermaphroditic, and this form of hermaphroditism he termed rudimentary protogynic. Schmitt-Marcel ('08) made microscopic examinations of the sex glands of a large number of *Rana temporaria* in different stages after metamorphosis and concluded that all the intermediates or hermaphrodites became males. Kuschakewitsch ('10) worked on *Rana esculenta* and



came to the conclusion that, if intermediates were found in a culture, all the individuals were intermediates and consequently some developed into males and some into females.

Witschi ('14) found that individuals of *Rana temporaria* that had just metamorphosed showed all the intermediate conditions between pure males and pure females. He believed that the testis developed out of an ovary and not from an undifferentiated gland. No evidence was found that an ovary developed out of a testis; for, usually, when a germ gland had begun to develop in the male direction, the whole or at least a part of it became male.

Witschi has advanced the idea that a germ cell in a frog becomes a male or a female cell according to the length of time it remains in the germinal epithelium. He found that when eggs developed under optimum temperatures ( $21^{\circ}\text{C}.$ ), no hermaphrodites appeared in the cultures; but when the eggs developed under low temperatures ( $10^{\circ}$  to  $15^{\circ}\text{C}.$ ) and under high temperatures ( $27^{\circ}\text{C}.$ ), intermediates were formed. Witschi concludes that heat and cold increase the chances of an early migration of some of the germ cells out of the germinal epithelium into the sex cords, and that when a part of the germ gland has thus once differentiated in the male direction, the whole gland usually changes into a testis, although the germ cells which remain in the germinal epithelium transform into oocytes. Under this assumption there would naturally be more males formed at either extreme of temperature. In the same way Witschi assumes that overripeness of the egg at fertilization so alters the trophic condition of the whole organism that only males are produced.

Abnormal temperatures and overripeness Witschi speaks of as 'Aussenfaktoren,' and claims that they may influence the sex of the organism. Under normal conditions they are absent and sex is then determined by 'Erbfaktoren' and 'Innenfaktoren.' The latter he speaks of as female determining: "Wenigstens spielen männchenbestimmende Innenfaktoren keine auffällige Rolle." These factors are local conditions in the germ gland which retard the migration of the germ cells into the sex cords. The 'Erbfaktoren,' however, are supposed to be the chief sex

determining factors under normal conditions. In attempting to explain how these factors operate, Witschi has adopted the interpretation of Goldschmidt ('12) that the female is homogametic with respect to sex (MFMF), while the male is heterogametic (MFMF'). In these formulae the letters are ranked in value as follows:  $F > M$ ,  $F > F'$ , and  $M > F'$ . Goldschmidt speaks of a variation in values of the factors as 'Potenzgraden,' which may be represented by figures; for example,  $M = 40$ ,  $F = 60$ , etc. It is assumed that these potencies may vary for different fertilized eggs and that, in order that the resulting offspring may become a male or a female which is free from the characters of the opposite sex, the dominance of one sex tendency over the other must reach a certain epistatic minimum. If it falls below this minimum, intermediates are formed.

Witschi found also, as had Pflüger, that different races of frogs varied as to the number of intermediates produced. Races of *Rana temporaria* from northern Germany (Königsberg) and from the Alps (Ursprungtal) differentiated early and only a few or no intermediates were produced. In middle Europe (Utrecht, Munich), however, intermediates were commonly formed. Witschi designates these races as differentiated races and undifferentiated races.

Considerable space has been devoted to the results obtained by Witschi, for it is about the only experimental evidence we have that an external factor may influence the resulting sex of the individual. There are other cases in which the sex of an individual appears to be reversed by factors influencing the egg before fertilization, such as overripeness of the eggs, overwork in reproduction, desiccation of the eggs before fertilization, etc. Some of these cases will be discussed later.

f. Discussion. It has not been found practicable to test sex determination in the lamprey by experimental means, similar to those employed in the case of the frog, because of the length of the larval period and the difficulty, under laboratory conditions, of rearing the larvae through the period of sex determination. Witschi thinks that in the early phylogenetic history of the frogs the germ cells were probably all of the same value as to sex and

that sex was determined wholly by external factors. In the lamprey it appears that the two sex potencies are almost balanced and that under normal conditions it is a matter of chance which sex develops, so that slight changes in the environment might suffice to throw the balance in favor of one or the other sex. It cannot be denied, however, that the eggs, from the time of fertilization, may show a greater tendency in favor of one sex than the other, as has been supposed by Witschi to be the case in normally developing eggs of the frog; but this inherited tendency may not be strong enough to prevent the formation of a series of intermediate individuals with glands ranging from those with no oocytes to those with no cysts. Under such conditions, it is not difficult to understand how a sex reversal might take place as a result of extraordinary external conditions. The more equally balanced the sex potencies are, the more easily a sex reversal might be effected.

Granting that sex potencies may be inherited factors, it does not seem necessary to assume that one sex is homozygous for sex and the other heterozygous, as Witschi and others have assumed, who felt themselves obliged to bring the phenomena of the inheritance of sex in line with those of the inheritance of mendelian characters. Before entering upon this question further, it will be necessary to summarize briefly the morphological evidences obtained from the present study in favor of a possibility of sex reversal in the lamprey.

Following an earlier indifferent period, when no sex characters are present, there is an indeterminate period in the early larval life of the lamprey during which the future sex of the individual cannot be determined, in spite of the fact that the sex characters are present. During this indeterminate period, all of the germ glands develop oocytes in greater or less number, with the exception of possibly a few in which no oocytes are found. At the same time many germ cells in all the glands remain in an indifferent condition and are found either as individual cells or in smaller or larger cysts. Since the secondary sexual characters do not appear until later in the life of the animal, there is during this period no other sex distinguishing character than

the presence or absence of oocytes. The presence of large cysts of indifferent germ cells has been taken to be a male character, the whole cyst being homologous to an oocyte with its follicle; but to a certain extent the male character remains obscure, since it is not until the animal approaches the sexually mature condition that the male germ cells can be identified as such. The presumably male germ cells of the larger cysts continue to divide until after metamorphosis, but the cells resulting from each division are, for a long time, not essentially different from the primordial germ cells. The only secondary characters that distinguish the adult male are the long, slender urogenital papilla and the absence of an anal fin, but these do not appear until after metamorphosis. Since this study does not involve the stages in which secondary sex characters are present, we are concerned only with the primary ones, namely, the presence of male or female sex cell. Only the latter are structurally recognizable in the stages studied, and they give us the only definite clue to the sex condition of the young larvae.

Oocytes appear in practically all lamprey larvae; in normally dioecious species oocytes appear only in about one-half of the young, while in the other half only male cells appear. Whether or not male germ cells occur in all lamprey larvae cannot now be stated for reasons already presented. When a germ cell, however, shows no tendency to transform into an oocyte, but continues to divide and form cysts, it has been assumed that it is potentially more strongly male than female in constitution. The activity of the germ cells may be along either of two lines. In the one case their tendency is toward growth, and in the other toward rapid multiplication. This difference in the activity of the cells may be due to an inherent tendency in the cells themselves or to factors operating in the cell environment. If due to the former it must be admitted that all the germ cells of the same gland do not have the same make-up and that during the process of development each cell inherits a different constitution after each division. Some of the cells are endowed with a tendency toward rapid division and others toward an early cessation of division and entrance upon a period of growth. It may be due to an unequal

partition of chromatin material during cell mitoses or to an unequal distribution of cytoplasmic material during the early divisions of the cells. Just as the germ cells are set aside from the somatic cells in early stages of development by some differences in their make-up, so the germ cells may also differ among themselves in their inherited structure after each division. There is direct proof that there is an unequal distribution of material among the cells in early stages of some animals, and that certain cells are destined to form certain parts of the body. These differences appear to be cytoplasmic in most cases; for example, in the case of *Cynthia*, in which (Conklin, '05) there are several kinds of organ-forming substances which are unequally distributed among the cleavage cells. In later stages, however, there is no direct proof of an unequal division of the cells; so in the case of the germ cells of the lamprey it must remain an assumption that unequal division does take place; but this would explain the two types of behavior of the germ cells.

Again the germ cells might all be assumed to have the same inherited structure, and yet they may develop along different lines on account of their different local environment in the gonad. In this case the behavior of the cells would be the result of factors or circumstances acting from without. These factors may be supposed to be differences in nutrition, the presence of various enzymes and toxins, differences in pressure, and various other factors operating in the germ gland of the animal. In this case it must be assumed that the germ cells are so constituted that they can respond to environmental factors in two different ways. Under certain conditions the cells will continue to divide, under others they will stop dividing and enter upon a period of growth.

As yet we know too little about the physiology of the cell to be able to decide between the two possibilities. We know that in a form like the lamprey, whether it eventually becomes a male or a female, the two kinds of cells make their appearance, some with a tendency toward rapid division and some with a tendency for growth. Now, since the latter is an undoubted female quality and the former is supposedly a male quality, practically every individual must possess both male and female potencies. That

these potencies are practically in a balanced condition is seen from the fact that both male and female sex cells appear in the majority of larvae. Sometimes a larva is inclined more strongly toward the female side and at other times it leans toward the male side. In some cases it appears that a larva may fluctuate back and forth between the two extremes until finally one or the other sex condition takes the lead and sex reversal becomes more difficult. This is indicated by the fact that the sex glands of older larvae from the period of sex differentiation (larvae 50 mm. to 70 mm. long) often show that an earlier sex condition has been replaced by that of the opposite sex. After sex has become definitely established, one or the other sex potency becomes so strong that only unusual circumstances are able to reverse the condition. The elements in the body or in the germ gland, which have specialized in the opposite direction, stop developing and either degenerate or remain in an undeveloped condition during the whole lifetime of the animal. The cysts in the developing ovary contain small cells which soon degenerate and disappear so that the larva soon becomes apparently a pure female. In the developing male gland the undeveloped oocytes remain, in many cases, even up to the adult stage; but often they degenerate in early stages, so that fragments of oocytes occur in the developing testes. Out of the juvenile hermaphroditic condition, therefore, both males and females eventually emerge.

The condition in the lamprey is not essentially different from that in *Myxine*, except that in the latter the two kinds of germ cells develop in different parts of the gonad, while in the lamprey there is no segregation of the two kinds of cells. The whole gland is in fact hermaphroditic in the lamprey while in *Myxine* the anterior portion of the gonad is ovarian and the posterior portion testicular. In some individuals of *Myxine* there is a tendency for the two kinds of cells to be mixed. This is especially true on the border-line between the testicular and ovarian portions. There is also probably no essential difference between the condition in the frog and that in the lamprey, and an explanation of the phenomena in one case should hold for the other as well. Whether or not different races of lampreys show a greater or

less tendency toward juvenile hermaphroditism is not known, and no opportunity has yet been offered for an investigation of this question.

What it is that keeps a larva with hermaphroditic tendencies from developing into a functional hermaphrodite is not known. It appears that when one of the sex tendencies takes the lead, it prevents the development of the structures characteristic of the other sex; or it may be that when one set of sex elements begins to degenerate, there are removed certain influences that have previously inhibited the development of the other set. Something similar to this is seen in most true hermaphrodites, where only one set of sex cells develops at a time, so that the animal is either protandrous or protogynous. In this case, too, the development of one set of germ cells is antagonistic to the development of the other, but a reversal always takes place when one group is exhausted. This may be due to the fact that certain hormones are eliminated from the germ cells which have taken the lead in development, and that these are unfavorable to the development of the other set. As soon as the first set of cells has been eliminated, a reversal takes place and the opposite set develops. It may be looked upon as alternate periods of vigor and depression as far as the particular germ cells go. In bisexual animals with juvenile hermaphroditic tendencies, it may be supposed that the animals never recover from the state of depression relative to the opposite sex.

Reviewing the case of the lamprey, the evidence seems to warrant the conclusion that sex is not irrevocably fixed at the time of fertilization; that the future sex of the animal is not definitely determined until the larva has reached a considerable size, and that sex is not the result of any unchangeable sex quality present in the egg at the time of fertilization, but is rather the outcome of a balanced sex potency which results in one or the other sex being formed, largely as a matter of chance under normal environmental conditions. It is possible that one sex potency may be stronger than the other from the beginning of development, and that even the germ cells themselves at the time of fertilization may be inclined in one or the other direc-

tion; but such an admission is not necessary for an explanation of what actually takes place.

Practically the same conclusion has been reached by Shull ('11) in the case of plants. He says:

May not maleness and femaleness be thought of as alternative states which can be crudely analogized with the acidity and alkalinity of chemical solutions. . . . In some species the sexes appear to represent a much more strongly polarized (?) condition than in other species, and a transition from the characters of the one sex to those of the other is attained only with the greatest rarity, if at all; while in other species the sex conditions may be so nearly balanced or neutral that individuals are not absolutely determined in their sex relations by their genotypic nature. . . . With such a conception of sex, it also appears probable that sex may be influenced sometimes by external factors as well as by internal ones, and in this case the preponderance of one sex over the other, which has been observed in many animals and plants, need not be attributed alone to selective disorganization of germ cells, a selective fertilization or a selective death rate, but might conceivably be controlled to a certain extent by environmental conditions, acting at some particular 'sensitive' period in the ontogeny of the organism in question (pp. 363-364).

4. *Present status of the sex problem.* We may now ask whether or not the view expressed above can be brought into harmony with current opinions concerning sex determination. The generally accepted view is that sex is established at the time of fertilization as a result of the presence or absence of so-called sex chromosomes in the fertilized egg. This view was first expressed by McClung in 1902. During the progress of his work on the maturation of the germ cells in insects, he found a certain body in the spermatocytes which was interpreted as being a sex-determining element. This body had been seen before by Henking ('91), Montgomery ('98), and Paulmier ('99), but it had not been suspected that it might be a sex-determining factor. McClung's statement concerning the function of the accessory chromosome as it was called, was as follows: ". . . it is the bearer of those qualities which pertain to the male organism, primary among which is the faculty of producing sex cells that have the form of spermatozoa." This interpretation was quite generally accepted. Previous to this time numerous theories had been advanced concerning the cause of the appearance of



two kinds of sexual individuals, each theory to be replaced by others, which further research found equally untenable. Investigators now began to search for this odd element in the sex cells of various species of animals. More and more forms, especially among insects were found in which the odd element was present in the germ cells and in which it became distributed to half of the mature cells.

It is natural that this discovery should have led to a qualitative explanation of sex. There apparently was something present in half of the male germ cells which, after fertilization, was responsible for the development of a male. This was McClung's interpretation and this explanation was accepted by the majority of his immediate followers.

The early work was done on the accessory chromosome of the male germ cells alone. When cytologists began investigations upon the chromosomal structure of the female germ cells (Wilson, '05; Stevens, '05, and others) it was found that this odd element was present there also, not singly but in duplicate. These two accessories were so distributed during maturation that every egg retained one, and consequently all the eggs were alike in their chromosomal structure. Theoretically, therefore, an egg which happened to be fertilized by a spermatozoon containing the accessory chromosome would give rise to a female, and not to a male as had been supposed to be the case. It became clear that the accessory chromosome could not be sex determining by virtue of any qualities it might possess, but rather that sex was due to a quantitative difference in the amount of the odd chromosomal material present in the fertilized egg.

Certain studies in heredity have shown that some characters are sex-linked. The interpretation of this fact is that the factors for such characters are carried by the sex chromosome. It was discovered that the inheritance of sex-linked characters in forms like moths, butterflies, and birds was such as to necessitate the assumption that the ova in these forms rather than the spermatozoa were dimorphic in regard to the sex chromosome. Later it was discovered by Seiler ('14) that there are actually two kinds of eggs in the moth *Phragmatobia fuliginosa*. In the case of

birds the problem has not yet been cleared up. Guyer thinks he has evidence that the spermatozoa are dimorphic in this form, while the inheritance of sex-linked characters in birds points to the egg as being dimorphic. In his last paper on the subject, Guyer ('16) again emphasizes the fact of the presence of two kinds of spermatozoa in the common fowl, but admits the possibility of only one kind being functional. If it be admitted that the eggs also are dimorphic, it would be difficult to explain why two kinds of cells should be produced in both sexes of the offspring.

The assumption of a dimorphism of both spermatozoa and ova of the same species has been made before. Castle ('03) proposed a theory of this sort. Such a theory necessitates the further assumption of selective fertilization, for which there is apparently no direct evidence.

In a recent paper by Stockard and Papanicolaou ('16), dealing with the hereditary transmission of degeneracy and deformities in alcoholized guinea-pigs, a statement is made that the junior author is in possession of data which indicates that the female guinea-pig, as well as the male, shares in the determination of sex, and that in this species both ova and spermatozoa may be dimorphic. Previous to this, Papanicolaou published some of his results in *Science* ('15), where he states that the sex of the guinea-pig is determined, sometimes by two and sometimes by three factors, depending upon whether or not the mother had previously given birth to young. The three factors are: 1) The sex tendency of the father; 2) the sex tendency of the mother; 3) the change of sex tendencies in the female from litter to litter. If these observations prove to be correct, the sex potency of the fertilized egg is not determined by a sex chromosome, unless there be a selective fertilization that is subject to variation according to the physiological condition of the parent.

An accessory chromosome has not been found in all forms studied. This does not, however, exclude the possibility of its being present, since it appears that it may often be united with some other chromosome. This seems to be the case in *Ascaris megalocephala* among invertebrates and *Necturus maculosus*

among vertebrates. If it occupy such a position, it might very easily escape observation. In plants no accessory chromosome has been found, except in *Salamonia biflora* in which Cardiff ('06) describes one, but his interpretation has been doubted by Strasburger and others. It is in insects that the accessory chromosome has been studied with most care, and in this group it has also been found that the secondary sexual characters apparently develop independently of the sex glands. It has been shown by Kellogg ('04), Meisenheimer ('09), Kopec ('11), and Steche ('12) that in moths, at least, the presence of a particular germ gland in the animal is not responsible for the development of the secondary sexual characters. In this case the primary and secondary sexual characters seem to develop in consequence of the presence in the developing embryo of a common factor or set of factors which may be located in sex chromosomes.

Many of the bodies that have been described as accessory or sex chromosomes are probably something else. Our knowledge concerning many of the cytoplasmic bodies in the cell is very limited, but it is known that some of them may occur among the chromosomes during mitosis. It will be recalled that Wilson ('13) in his work on *Pentatoma* warned against mistaking a so-called chromatoid body in certain cells for a sex chromosome. Wodsadelek ('14) found a similar body in sex cells of the horse, and Bachhuber ('16) found it in the rabbit. There also seems to be a certain relation between the nucleolus of the cell and the accessory chromosome in certain cases. Goldsmith ('16) thinks he has found evidence in *Pselliodes cinctus* that the nucleolus is composed of both chromatic and achromatic material. The achromatic material he thinks is linin, or closely related to it in composition, while the chromatic part constitutes the sex chromosome.

It must be admitted, however, that an accessory chromosome is undoubtedly present in the cells of a great number of forms, and that it may function as a sex determiner, at least in the absence of other factors. There is, however, good reason for believing that it forms only one link in a series of events that precede the development of sex. This conclusion has also been

reached by Doncaster ('14) who says: "It seems evident that sex cannot depend on a chromosome alone for the chromosome must act by its relation with the cell-protoplasm and it is on this relation that sex determination depends." This same proposition is admitted by Morgan ('15). He says: "It is quite conceivable that one or more of these other factors might so change that the sex differentiation would become inoperative or even change so that these other factors themselves become the differentiators that determine sex" (p. 95). He admits that the environment is one of the important factors that enters into the development of every individual and that it is quite possible that it may turn the scale and determine sex. Loeb ('16) accepts the cytological evidences for sex determination by sex chromosomes, but speaks also of a physiological basis of sex determination by specific substances or internal secretions. He thinks it possible that the sex chromosomes may favor the formation of specific internal secretions which are responsible for the formation of sex characters in the animal and that if it should be found "possible to modify secretions by outside conditions or to feed the body with certain as yet unknown specific substances the influence of the sex chromosome upon the determination of sex may be overcome" (p. 228).

From these statements it will be seen that the possibility is admitted by some of the foremost investigators of the sex problem that all germ cells carry the potentialities of both male and female, and that after fertilization the egg may be inclined in one or the other direction, but not so strongly that it excludes the possibility of a reversal in the other direction. There seems to be at the present time a decided tendency away from the idea that the sex chromosomes carry absolute sex determiners. We are, therefore, no longer antagonistic to the idea that other sex factors may exist, either in the cell itself, in the developing organism which comes from the germ cell, or in the environment of the cell or organism.

5. *Discussion of the hermaphroditic condition found in the lamprey in connection with other sex phenomena, not easily explained by current theories.* a. Normal hermaphroditism. The sex-

chromosome hypothesis does not offer a satisfactory explanation of normal hermaphroditism in animals and plants. Hermaphrodites normally produce both male and female sex cells in the same individual, but in most cases the two kinds of cells are not matured at the same time. Usually the male germ cells are ripened first, and in such cases the species is known as protandric. When the eggs are matured first, the species is known as protogonic, and when the male and female germ cells are produced at the same time, the condition is known as simultaneous hermaphroditism. The latter condition is usually found in species with more or less widely separated male and female sex glands, but it may also appear in species in which an ovotestis is found, as, for example, in certain pulmonates. When the germ cells are ripened during successive seasons of the life-cycle of the animal, the condition may be called polycyclic. On the other hand, if the animal produces only one kind of germ cells during the early period of its life and the other kind of germ cells during the later period, the condition may be termed monocyclic hermaphroditism. The latter condition exists in *Crepidula fornicata*. Orton ('09) has made a study of this form and has found that the individuals associated in chains offer transitional series from maleness to femaleness both in primary and secondary sexual characters, beginning with a male in the young stage and ending with a female in the older stages. Three hundred and fifty chains were examined, and it was found that the individuals could be arranged as follows: 1) male; 2) male with rudimentary uterus; 3) hermaphrodite with small uterus; 4) hermaphrodite; 5) hermaphrodite with small penis; 6) female with rudimentary penis; 7) female.

In monocyclic hermaphroditism it appears that with the aging of the animal its metabolism becomes antagonistic to the development of one or the other of the two kinds of sex cells. In the case of *Crepidula*, the metabolism of the young animal is favorable to the development of the male sex cells, while the metabolism of the older animal is more favorable to the development of the female sex cells. In the case of polycyclic hermaphrodites, when the two kinds of germ cells are ripened in close succession,

the development of the second kind of germ cells may be the result of a changed metabolism.

In some true hermaphrodites sex conditions seem to be disturbed at times so that true males and females appear. According to Maupas ('00), the number of males per thousand females in various nematode worms may vary from 0.13 to 45.

b. Alternation of the hermaphroditic and the dioecious condition. A more complex case of hermaphroditism than those mentioned above is that found in the nematode worm *Rhabdites* (*Rhabdonema*) *nigrovenosum*, which is parasitic in the lungs of frogs. While in the lungs, the worms are hermaphroditic, but in the free-living state, which alternates with the parasitic, two sexes occur. The free-living worms again give rise to hermaphroditic parasitic offspring. This has been explained by Boveri ('11) and Schleip ('11) as being due to the disappearance of one kind of spermatozoa in the free-living males, so that upon fertilization only one kind of sexual individual is produced, namely, a female which again becomes parasitic. This female is capable of giving rise to both spermatozoa and eggs, both of which should have the same chromosomal make-up. During maturation three kinds of spermatids are produced, with five, six, and seven chromosomes, respectively. It is supposed that the last kind degenerates.

Some evidence has been advanced for a chromosomal explanation of true hermaphroditism. Zarnik ('11) thinks that in certain hermaphroditic Pteropods, the female cells are of one kind only (homogametic); while the male cells are of two kinds (heterogametic), but that only one kind of male cells is functional, namely, the one corresponding in chromosomal make-up to that of the female cells. The offspring from such union should result in a female, but instead it develops into a hermaphrodite in which again half of the male germ cells degenerate.

Krüger ('12) found what she thinks is an accessory chromosome in the hermaphrodite *Rhabdites* *aberrans*. During spermatogenesis it becomes distributed equally among the spermatozoa, with the exception of a very few cases when it lags behind and is retained in one cell. Apparently in this species the spermatozoon

simply initiates development in the egg and the sperm nucleus degenerates. The parthenogenetically developing egg forms a hermaphrodite. Only in one case was a fusion of the male and the female nucleus observed, and Miss Krüger assumed that the male nucleus in this case was one in which the accessory chromosome was lacking. This would give rise to a male of which there were a few formed.

Demoll ('12) thinks that in *Helix pomatia*, which is hermaphroditic, two kinds of spermatozoa result from the unequal distribution of the sex chromosome and that only one kind, that with the accessory, becomes functional.

It appears from the above cases, namely, that of the gastropods, which are true hermaphrodites, and that of Rhabdites, in which the hermaphroditic condition alternates with the dioecious, that sex cannot be the result of the action of the sex chromosome alone, but that the activities and behavior of the sex chromosomes which results in their peculiar distribution must be due to some physiological activity in the cell which antedates the sex chromosomes, so that the latter are simply the final link in a series of processes which determine the sex potentiality of the cell. This possibility has been admitted by Schleip in the case of Rhabdites. He says: "Es scheint, dass die Entwicklung mancher Keimzellen zu Spermatozyten statt zu Oozyten zum Teil auf Ursachen beruht, die ausserhalb dieser Keimzellen liegen." . . . "Diese äusseren Ursachen brauchen nicht ausserhalb desselben befinden; man kann sogar vielleicht daran denken, dass innere Secretion dabei eine Rolle spielt" (p. 128). Further on Schleip adds that external conditions may influence the development of the sex cells. He says: "Wie bei manchen Tieren äussere Bedingungen einen Einfluss auf das Geschlecht der sich entwickelnden Tiere auszuüben imstande zu sein schienen, so beeinflussen also äussere Bedingungen bei der zwittrigen Generationen die Entwicklungsrichtung der Keimzellen." He says further: "Daher wird die Frage erlaubt sein, ob die verschiedene Chromosomenzahl überhaupt einen Einfluss auf die Geschlechtsbestimmung hat, und ob die Spermien nicht aus anderen Ursachen und in anderer Weise in männliche und weibliche dif-

ferenziert sind und die verschiedenen Chromosomenzahl, die sie erhalten, nur die Folge davon ist."

Another line of investigation on hermaphroditism is the study of the segregation of the germ cells in the sex gland. In the case of *Sagitta*, Elpatewsky ('09, '10) finds a body in the cytoplasm of the cells during early cleavages, which he calls the 'besondere Körper.' This is retained by only one cell after each cleavage up to the sixth, when it divides during mitosis and part of it passes to each of the resulting cells. These two cells become the germ cells, and Elpatewsky believes that one becomes the forerunner of the spermatozoa and the other of ova, and that the former gets a larger portion of the 'besondere Körper.' Ancel ('03) has worked on the early development of the germ cells in *Helix pomatia* and thinks that three kinds of cells appear in the germ gland, spermatozoa, oocytes, and nurse cells. He thinks that the primordial germ cells become transformed into female and male elements according to whether or not the nurse cells are present at the time of transformation. Buresch ('11) thinks that in *Helix arbustorum*, also, the fate of the indifferent germ cell depends on its proximity to a nurse cell. The cases which have been cited, show that the differentiation of the germ cells into male and female cells has been interpreted as being due to nuclear differences in some cases, cytoplasmic differences in others, and to differences in the environment of the cells in still other cases. If we conceive of sex as a metabolic state rather than the result of definite sex factors, it is easy to see how any one of the above factors might result in a metabolic change which would throw the balance in favor of one or the other sex.

It is unfortunate that so little work has been done on the history of the germ cells in hermaphroditic animals; for it is in these forms that one undoubtedly must look for valuable clues to the problem of sex determination.

c. The effect of delayed fertilization on sex. An interesting case in which sex metabolism seems to be disturbed by outside factors is that of the frog in which delayed fertilization results in the development of the eggs into male individuals exclusively. It was found both by R. Hertwig ('05, '06, '07), and by Kusch-



kewitsch ('10) that the percentage of males increased with the length of time that fertilization was delayed. Kuschakewitsch found that when fertilization was delayed as much as eighty-nine hours all of the eggs developed into males. The mortality among all the eggs in the culture was about 4 per cent. In the case of the frog, an accessory chromosome has been described, both by Levy ('15) and by Swingle ('17). Levy found twenty-five chromosomes in the male germ cells of *Rana esculenta*. During maturation division these were so distributed that half of the cells received twelve and the other half thirteen chromosomes. The odd chromosome of the thirteen is the sex chromosome. Levy believes that the accessory chromosome undoubtedly has something to do with sex, but he thinks that it is not the only sex-determining factor. He says: "Man darf aber die Geschlechtschromosomen nicht als den geschlechtsbestimmenden Faktor bezeichnen, den sie sind nur die zuerst morphologisch erkennbaren Zeugen einer stattgefundenen sexuellen Differenzierung."

Swingle found the spermatogonial number of chromosomes to be twenty-five in *Rana pipiens*. He found some cases in which the sex chromosome divided during the second spermatocyte division instead of during the first, and one case in which the two parts of the X-body were unequal in size. He thinks that there may be some connection between the abnormality of chromatin distribution which results, presumably, in the production of three kinds of spermatozoa, and the fact that in certain strains of the species, males, females, and individuals possessing marked hermaphroditic tendencies occur.

In the case of the frog it seems evident, both from the experiments of Hertwig and Kuschakewitsch on delayed fertilization and from those of Witschi ('14) on the effects of temperature on the sex of the animal, that the accessory chromosomes, known to be present, are not the sole sex determiners. Such a conclusion is not a condemnation of the sex-chromosome theory. If other factors also affect the sex of an individual, it shows that the sex chromosome is but one of many such factors which may bring about the same result. Temperature, for instance, may result

in reactions in the protoplasm of the cells or may so change the whole metabolism of the organism that the visible results might be quite different. An analogy may be drawn between the phenomena of sex and those of the red-flowered *Primula* which, according to Klebs ('03) becomes white when grown at high temperatures. In this case the two color potencies are present in the organism, and which one shall appear depends upon an external factor, namely, temperature. Similarly, sex potencies may assert themselves differently under various conditions, so that a reversal may take place, or intermediates be formed, such as are found in cyclostomes and amphibians, and possibly in many fishes as well.

d. Hermaphroditism and sex reversal due to external conditions. Another interesting case showing the double sex potentiality of early larvae is recorded by Baltzer ('14). He found in *Bonellia viridis*, the males of which live parasitic upon the females, that if the larvae have a chance to attach themselves to a female they become males, and if they do not succeed in becoming attached they form females. If they are allowed to attach themselves and are later removed, they become hermaphrodites. In the attached larvae the sex determining substances are undoubtedly taken up from the host, since the female-determining substance seems to be stronger in the free-living state. Baltzer concludes that sex is partly predetermined and partly epigenetic, and that both sex tendencies are inherited but in different degrees. He believes that the male tendency is stronger than the female. If this be so, we have here a case of sex reversal, providing the larva remains unattached. A case somewhat similar to that of *Bonellia* is that of the protandric hermaphrodite *Crepidula plana*, in which Gould ('17) finds that the development of the male phase is dependent upon the presence of a larger individual of the same species, but not necessarily a female. In the absence of a larger individual, the larva develops into a female, but the process of transformation in the female direction may be halted at any time, up to the period of formation of growing oocytes, by bringing the animal into proximity with an older individual. Gould does not offer any explanation as to the nature

of the stimulus exercised by the older individual over the sex of the larva.

Among plants there seem to be many cases which indicate that every individual possesses a double sex potentiality. It appears that in some of the lower types of plants which are normally dioecious, the organs of the opposite sex can be made to appear on all the individuals under proper culture conditions; that is, the male plant will produce female organs and vice versa.

Bordage ('98) cut back the apex of young male plants of *Carica papaya* just before the appearance of the first male flowers. Lateral branches arose below the cut, and these produced female flowers and fruit. Strasburger ('00) found that the smut *Ustilago violacea* caused the dioecious plant *Melandryum album* to produce the opposite sex organs; that is, the male organs appeared on the female. The pistils remained undeveloped, while the normally rudimentary anthers grew large and produced pollen mother cells. Later, Strasburger ('09) came to the conclusion, from a consideration of many evidences, that sex determination in plants cannot be the result of mendelian segregation. He says: "Ich bin nach alledem der Ansicht dass alle Versuche, die Geschlechtsbestimmung getrenntgeschlechtlicher Organismen auf Mendelische Spaltungsregeln zuruckzuföhren, erfolglos bleiben werden" (p. 17). Strasburger also did not consider the so-called sex chromosomes as true chromosomes. He says: "Denn nicht nur zeigen sie ein eingeartigten Verhalten, sondern auch ihre Beseitigung aus den Geschlechtszellen ist möglich, was für Träger von Erbeinheiten nicht zulässig wäre." Should they be proved to stand in some relation to sex, they might yet be individual linin bodies "die aber nicht Pangene föhren, sondern der Aufnahme des über das Geschlecht bestimmenden Stoffes dienen" (p. 22).

It may be suggested in this connection that other bodies are present in the cell which may become unequally divided during mitosis. This is true of plasmosomes, which may not always dissolve and become diffused throughout the cell before division. It may be equally true of mitochondria and other cytoplasmic bodies. Such an hypothesis has been advanced by Schaudin

('05) in the case of Protozoa. A normally functioning cell is regarded by him as a hermaphrodite which has the male and female qualities equally balanced. The differentiation which leads to the formation of gametes is due to inequalities of cell division which result in a more or less imperfect distribution of the qualities of the parent cell between the daughter cells, so that some cells may receive more male and others more female properties. The male cells show greater kinetic energy; the female cells greater trophic energy. The opposite tendencies accumulate in different cells which thus become one-sided in their vital activities. The want of balance may reach a stage in which syngamy must take place or the cell dies.

A similar idea was advanced above, in my discussion of the appearance of two kinds of germ cells in the sex glands of the lamprey. In this case, too, the development of the two kinds of cells in the same gland may be due to a disturbance in the metabolism of the cell during mitosis, which results in the development of a cell along either one or the other of two potential lines. It is conceivable also that there may be various grades of male and female potentialities in the germ cells thus formed, and that even in their mature condition some cells may be more strongly sexed than others. After fertilization, the same differences of sex potentialities may exist, and, in so far as no other factors are introduced to disturb the relative sex potentialities, the sex of the resultant animal may be said to be determined at the time of fertilization.

Whether or not these differences in sex potentiality are the result of a variation in the chromosomal make-up of the cells is not certain. This suggestion appears contrary to certain known facts of sex-linked inheritance, which seem to require for their interpretation that the sex characters reside in the same chromosome as the sex-linked character. It might be assumed equally well, however, that certain characters appear, only when associated with a certain kind of cell metabolism which may be peculiar to one or the other sex. This conception might also account for the exceptions to the inheritance of sex-linked characters which are difficult to explain by the chromosomal theory.

Some further examples of mixed sex among plants may be given. Among the flowering plants some species are hermaphroditic, others dioecious, and still others produce three kinds of individuals, namely, males, females, and hermaphrodites, as, for example, the sweet pea. In the strawberry three kinds of flowers are produced, staminate, pistillate, and perfect. Valteau ('16) has investigated the inheritance of sex in grapes, and his results are as follows: The wild grape develops two kinds of individuals, staminate and pistillate, and both possess flowers of the opposite sex in a suppressed condition. The grape, therefore, occupies an intermediate position between purely dioecious plants, like the willow, and purely monoecious plants, like the apple. On individual plants of the grape all gradations are sometimes found, from staminate to functionally hermaphroditic flowers, and sometimes only hermaphroditic flowers are produced. Certain clusters of the vine may be entirely staminate, while other clusters on the same vine contain all gradations from staminate to functionally perfect flowers. In the grape, therefore, both staminate and pistillate vines carry the determiners for femaleness and maleness, respectively, but with one or the other partially suppressed. Valteau draws the conclusion that, if the chromosomes carry the determiners for sex, then in hermaphroditic plants the determiners for maleness and femaleness must be carried in the same chromosome. There are two possibilities, therefore, for the origin of functional hermaphrodites. The maleness may express itself fully in one of the chromosomes bearing the determiners for femaleness in a pistillate plant and femaleness may express itself similarly in staminate plants.

Pritchard ('16) discusses the change of sex in hemp. Hemp is dioecious, and the female plant is distinguished by its dense foliage as well as by the production of female flowers. The male plants have very scanty foliage. The sex ratio is normally 1:1. Hermaphroditic individuals appear in small numbers, but they are of the female type and predominantly female in flower development. Disturbances in the plant's physiological equilibrium were induced by the removal of flowers and of vegetative parts, as well as by the injection of various chemicals into the stem.

It was found that sex was alterable by removal of flowers. Removal of female flowers caused staminate flowers to appear, and the removal of staminate flowers resulted in the development of female flowers. Pritchard believes that the change is probably due to disturbances in nutrition. He concludes that maleness and femaleness are not always fixed characters, but frequently appear more like responses of the developing organism to external stimuli. He thinks that facts do not support the theory that sex is wholly a matter of zygotic constitution, but indicate that both males and females are partially hermaphroditic.

Certain plants which, under normal conditions are true hermaphrodites, will, under other conditions, produce two kinds of sexual individuals. This is true of certain mosses and ferns which normally produce antheridia and archegonia on the same plant, but which, by being supplied with a certain kind of nourishment will produce only one or the other of the two kinds of germ cells. Again, it has been found that under certain conditions some dioecious plants may become monoecious. Wuist ('13) found that *Onoclea struthiopteris*, which is normally dioecious, could be induced to become monoecious under proper culture conditions, so that the male plant produced female organs and the female plant produced male organs. Here, again, the appearance of the organs of the opposite sex is apparently due to the nutritional environment.

*e.* Hermaphroditism as a result of hybridization. During the last few years some interesting facts have been brought out in connection with hybridization in animals and these seem to throw some light upon the sex problem. Goldschmidt ('16, '17) found that by crossing European and Japanese races of the gypsy-moth many so-called gynandromorphs were produced. Different results were obtained if the material had a different race origin. The explanation of this seemed to be that the potency of the sex factors differed in different races. It will be seen that this case is somewhat similar to that of the frog, in which Hertwig and Witschi found a racial difference as regards the tendency toward juvenile hermaphroditism; but in the latter case the hermaphroditic condition was not retained up to the adult stage.

It was supposed by Goldschmidt that the sex potency varied with the geographical distribution of the moth, and for this reason it was decided to study the behavior of different local forms of the Japanese moths crossed inter se and with European moths. The result was that a great number of individuals were obtained, which, for the various crosses, showed all intermediate conditions between true males and true females; consequently, if maleness and femaleness are represented as the end points of a series, say one as zero and the other as one hundred, a given moth might be represented by twelve, thirty-five, forty-two, etc. These animals do not represent a mixture of the primary and secondary characters of the two sexes, but a definite point between the two extremes, maleness and femaleness. Since the term gynandromorphism applies only to individuals showing a mosaic of characters of both sexes, Goldschmidt discards this term; for in the moths the entire individual represents a definite quantitatively fixed point intermediate between the two sexes, and not a mixture of the characters of both sexes. Such sex intermediates he calls intersexes—female intersexes, if they are genetically female, but transferred to some stage toward maleness, and male intersexes, if they are genetically male, but transferred to some point in the opposite direction. Goldschmidt has succeeded in breeding every step from a normal female through the different intersexes to a normal male; also the steps starting with the normal males and passing through the male intersexes toward the female up to three-fourths of the way. Every single step can be produced by the right combination of races. The change in any given direction is through the secondary characters first and the primary characters last.

The explanation of the above condition appears to be that each sex possesses the potentiality of the other. In both sexes, irrespective of the zygotic constitution, both anlagen may become patent; which one shall appear depends entirely upon the quantitative relation between the two potentialities. Applying symbols and recognizing that the female is heterozygous for sex in moths, Goldschmidt makes use of the following formulae:  $FFMm$  = Female,  $FFMM$  = Male. The value of the sex factors

he speaks of as a potency or valency. Now, it may be assumed that in a certain case the female factorial set, FF, has a value of 80 units, and the male factor, M, a value of 60 units. The formulae would then read as follows:  $\frac{FF}{80} \frac{Mm}{60} = \text{Female}$ ;  $\frac{FF}{80} \frac{MM}{60+60} = \text{Male}$ . In the first formula the female set overpowers the male set by twenty units, and in the second formula the male set overpowers the female set by forty. According to Goldschmidt, two possibilities are open. Either the slightest preponderance of one over the other, say only one unit, is sufficient to determine the male or the female sex, or there is a necessary minimum of preponderance beyond which only one or the other sex appears. This minimum he speaks of as the epistatic minimum. If the epistatic minimum be twenty; then when  $FF - M$  is greater than twenty a female is produced, while if  $MM - FF$  is greater than twenty then a male is the result. The intermediate points represent the intersexes and, if they are heterozygous for M, they are intersexual females, but if they are homozygous for M, they are intersexual males. Definite races possess special potencies for the male sex factors. A cross of races of similar potencies gives normal offspring. Races of different potencies of the male factors give female intersexes in the  $F_1$  generation if the mother belongs to a race of lower potency. The degree of intersexuality depends upon the differences in the potencies.

Another interesting case which seems to show that sex may be disturbed by hybridization is that of the Norway rat when hybridized with the albino rat. King and Stotsenburg ('15) found a great excess of males among hybrid rats and came to the conclusion "... that hybridization alters the sex ratio by producing a marked increase in the relative proportion of males" (p. 110). Detlefson ('14) on the other hand, found a marked preponderance of females, especially in the early hybrid generations of the wild Brazilian guinea-pig and the common domestic guinea-pig.

Riddle ('16, and others) has conducted an important series of experiments on sex behavior in crosses between the various races of domestic pigeons. This work was begun by Professor



Whitman and, since his death, the experiments have been continued by Doctor Riddle. Whitman found that, if certain distantly related pigeons were mated, for example, individuals of different families, only male offspring resulted. If matings were made of individuals not so distinctly related, as, for example, between different genera, and to this was added the element of overwork in reproduction, males only were produced in the early part of the season and females only in the later part of the season. He also observed that at the transition period during the summer some pairs of eggs produced males and females, the first usually male and the second female. It was noticed, further that toward the end of the season the eggs were not quite able to hatch, and produced embryos of fewer and fewer days' development. This led Whitman to conclude that the developmental energy is greatest in the male-producing season.

Riddle, in a long series of experiments, has been able to verify the results obtained by Professor Whitman. He has also discovered many more facts which tend to show that in pigeons there is a reversal of sex, and that under certain conditions male offspring are hatched from normally female-producing germ cells, and vice versa.

In birds there should be, according to evidence obtained from experimental breeding, two kinds of eggs; one maleproducing the other femaleproducing. These two kinds should normally be produced in equal numbers. Riddle does not deny the existence of a chromosomal difference in the eggs of birds. He admits that it has been definitely shown that in some species, at least, when bred under stable conditions, certain chromosomes are associated with sex; but he denies that the sole cause of sex lies in the sex chromosome and that sex is definitely fixed and non-reversible from the very beginning of development. Data collected, he says, "strongly indicates that the basis of sex is a fluid, reversible process; that the basis of adult sexual difference is a *quantitative* rather than a *qualitative* thing."

In pigeons, therefore, it has been shown that eggs which normally develop into males or into females can have their developmental energy so changed by the introduction of spermatozoa

from another species, or through overwork of the parent in reproduction, that they produce individuals of the opposite sex. Sometimes this sex reversal is not absolutely complete, for many of the females showed different grades of masculinity in their sex behavior. Females hatched from eggs laid earlier in the season were more masculine in their behavior than those of their own full sisters hatched later in the season; and a female hatched from the first egg of a clutch was more masculine than her sister hatched from the second egg of the clutch. Here there is, therefore, a second form of intersexualism which does not show in the primary sex characters, but in the sex behavior.

*f.* Sporadic hermaphroditism. Banta ('16) has published some observations on the appearance of sex intergrades in the parthenogenetic *Phyllopod*, *Simocephalus vetulus*. The culture was started from material collected in an outdoor pond and the propagation was continued in the laboratory. During the 131st generation of parthenogenetic offspring, one of the strains suddenly produced a large percentage of males, together with some normal females, and a large number of sex intergrades. These intergrades were either males, with one or more female secondary sex characters, or females, with one to several secondary male characters, together with some individuals which had hermaphroditic sex glands and showed various combinations of male and female secondary sex characters. The sex intergrades are of all possible sorts of combinations of secondary and primary sex characters. The highly male-like female intergrades produced few or no young, and males with one or more female secondary sex characters in nearly every case had incompletely developed reproductive organs.

Banta succeeded in propagating female intergrades for sixteen generations with no apparent change in the ratio of the various forms and with no apparent tendency of the stock to lose vigor or become less prolific. An attempt has been made to classify the intergrades on the basis of sex characters, and no less than twenty classes are distinguished. At the ends of the scale are the normal males and females.

Banta draws the conclusion from his observations that sex depends on environmental factors which influence the general physiological whole of the organism. In the intergrades the sexual balance has in some way been disturbed and the origin of this disturbance he considers a mutation.

*g.* Hermaphroditism as a result of hormone action. Another interesting case of disturbed sexual condition is found in the so-called free-martin in cattle. Frank Lillie ('16, '17) has made a study of this problem. Forty-one cases of twins were examined in utero and a classification made of them without a possibility of error. In fourteen cases both members were males, in six cases both were females, and in twenty-one cases the two were of opposite sex; 97.5 per cent were monochorial, but, in spite of this, nearly all were dizygotic as determined by the number of corpora lutea present. It was found that twins in cattle are nearly always the result of fertilization of an ovum from each ovary. As development proceeds, the developing embryos sink down into the median portion of the uterus and the blood-vessels anastomose in the chorion, so that it is possible to inject the blood-vessels of either foetus from the other. If both of the embryos are of the same sex, no harm results from the continuity of their circulations; but if of different sex, the reproductive system of the female is largely suppressed and certain male organs are developed. This is interpreted as a case of hormone action which may be due to a more precocious development of the male hormone or to its natural dominance. In this case, therefore, a distinction can be made between the effects of the sex-determining factors that are zygotic and those due to hormones. But the sex reversal is not complete and the result is the development of an intersexual individual.

*h.* Hermaphroditism as the result of parasitism. That a partial reversal of sex may be induced by parasitism has been observed by Geoffrey Smith ('10) in the case of the spider-crab, *Inachus*, when infected with the rhizocephalan, *Sacculina*. The males, under the influence of the parasite, are capable of assuming all the female secondary sex characters, and often even develop ova in the testes. In this case, however, the females do not seem

to develop toward the male line when infected with the parasite. The explanation offered by Smith is that the parasite causes the host to elaborate a yolk substance similar to that which is elaborated in the ovaries during growth of the eggs. The apparent change of sex, therefore, is due to a change in the metabolism of the organism. It is clear that such a change could take place without the assumption that one sex is heterozygous for sex and the other homozygous, as Smith has assumed. If the change in the sexual condition be due to a change of metabolism in the direction that he has suggested, it follows that only the males should take on the characters of the opposite sex.

i. Sex in parthenogenetic animals. The determination of sex in parthenogenetic animals has been studied by various investigators. Woltereck ('11), who worked on *Daphnia*, came to the conclusion that there are, in each egg, competing sex substances, one kind becoming active at the maturation of the egg, while the other remains latent. In summing up his results he says:

Die resultate meiner Versuche lassen sich nur verstehen, wenn wir in jedem Ei verschiedene konkurrierende Geschlechtssubstanzen annehmen, von denen jedesmal die eine aktiviert wird, während die andere latent bleibt . . . . die Geschlechtssubstanzen selbst können wir uns unter dem Bilde von (latenten) Profermenten und (aktivierten) Fermenten vorstellen.

In the rotifer *Hydatina senta*, A. F. Shull ('12) found that it is decided, in the growth period of the parthenogenetic egg from which the female hatches, whether it is to be a female-producer or a male-producer, or, in other words, that sex is determined a generation in advance. In some later experiments upon this form it has been found by Shull and Ladoff ('16) that an important factor involved in the production of male-producers is the amount of oxygen present in the culture, and that this probably acts by increasing the rate of the physiological processes taking place in the body. This conclusion is analogous to that arrived at by Riddle in connection with his experiments on sex in pigeons. Riddle says: ". . . the low-storage capacity of the male-producing eggs as compared with the high storage capacity of the female-producing eggs is therefore an index of higher oxidizing

capacity of the male-producing eggs as compared with the female-producing eggs."

More recently Whitney ('19) has reinvestigated the problem relative to the effect of oxygen as male-producer in the rotifer, and has come to conclusions opposite to those of Shull, namely, that oxygen does not act as a factor in the production of male-producers. The question cannot be considered fully settled.

*j.* Variations in sex ratio. A variation in sex ratio might indicate that sex is not irrevocably established at the time of fertilization. It has been found that, in certain dioecious plants, females are more commonly derived from seeds of one region and males from those of another region. This may be due to differences in the metabolic activity of the two kinds of seeds, brought about, possibly, by differences in the conditions of the environment under which they were raised.

Montgomery ('08) found that there were 8.19 males for every female in a count of 41,749 spiderlings. Out of the total of 127 cocoons, only eight showed a male ratio of less than one. Out of the total number of eggs in the cocoons only 2871 failed to hatch, and even though all of these should be assumed to be female eggs, the ratio would not be appreciably altered and the results cannot, therefore, be due to selective survival. Examples of this sort might be given by the score, and they are not easy to explain on the hypothesis that the chromosomes are the only and absolute sex determiners; for this hypothesis demands that there should be an equal number of males and females produced.

Pearl and Parshley ('13) have found that, in cattle, the sex of the offspring is somewhat dependent upon the time of coitus. Early in the heat the number of males to one hundred females was 98.4; in the middle of heat the ratio was 115.5, and late in heat it was 154.8. The conclusion is drawn that, granting the presence of an X-chromosome, the results may be interpreted by assuming that it is not a positive cause of sex differentiation, but rather an inhibitor of the development of male characters—two doses inhibits maleness, while one dose is insufficient. On this hypothesis it is assumed that the general conditions of metab-

olism in the germ cells might modify sex. The case is similar to that of delayed fertilization in the frog which results in the formation of an increased number of males.

In fish cultures it is not rare to find an excess of males or of females. Woltereck ('08) has reported various records made by Thumm upon the sex ratio in fishes. In *Jenynsia lineata* broods were obtained of 68, 92, and 116 individuals without a single female. A female of *Cichlasoma nigrofasciatum* three years old, bred to a male one year old, gave a progeny of 800 individuals, of which not fifty were females. A female of the same species one year old, bred to a male two years old, (the same male as in the first case), gave 400 young, of which over 300 were females. To summarize: "aeltere starke Weibchen, verpaart mit jüngeren, daher schwächeren Männchen, brachten in Nachzucht vorwiegend Männchen und umgekehrt." It was also found that in viviparous 'Körpflingen' the percentages of males were higher in the spring than later in the season, and that in the fall it was often very low. This apparently corresponds with the results of Riddle on pigeons, where also the percentage of males is greater in the early part of the season. It does not seem possible that the results obtained by Thumm in the cases above are due to selective survival.

6. *General conclusions in regard to the problem of sex determination.* In the case of the lamprey it has been seen that, in young stages, a series of individuals may be arranged exhibiting all the intermediate forms between apparently pure females and apparently pure males. Pure in this sense is used to designate the individuals which possess no visible characters which normally distinguish the opposite sex. Out of the sex intermediates both males and females develop, so that in the adult condition only two kinds of individuals are found, functional males and females. The designation of sex intermediates among the young is based on the appearance in the germ gland of primary sex characters, namely, oocytes and cell nests. The presence of oocytes in the germ gland is unquestionably a female character, while the presence of cysts may indicate a juvenile condition or a male character. The oocytes, in one case, and the well-developed cysts, in.

the other, may be considered homologous characters of the opposite sexes.

Judging from the quantitative appearance of the characters in the germ glands of hundreds of larvae which have been carefully studied, the conclusion is reached that every individual is a potential hermaphrodite possessing the sex qualities pertaining to either sex. It appears, however, that some individuals are more strongly inclined toward the male side, others more strongly toward the female side. Some, on the other hand, are apparently in a balanced condition as regards sex, and it would be impossible to say whether they are more strongly male or more strongly female. This condition usually lasts only a short time, after which one sex takes the lead over the other. When, in the course of time, an individual has become more strongly male or female, the opposite sex character gradually disappears, or at least remains undeveloped; so that if it appear at all in the adult, it is in a rudimentary or degenerate condition. This is the case, for instance, with the oocytes which are in an undeveloped condition in the adult testes. The secondary sex characters which appear in the adult are probably not hereditary characters at all, but are formed as a result of the presence of special kinds of hormones produced by the testis or ovary. These secondary characters are not present until after metamorphosis, when one or the other appears, depending upon the form of germ gland present.

As far as the primary sex characters are concerned, it appears that both male and female potencies exist in every individual from the beginning of development; that is, from the time that the egg is fertilized, and probably in both of the sex cells that are brought together in fertilization. These potencies then are transmitted from parents to offspring. It seems quite likely, however, that the two potencies are not always transferred from parent to offspring in equal strength, so that the two are not, in all cases, in a balanced condition from the very beginning. This appears from the fact that during the stage of sex differentiation all kinds of variations are found as to the quantitative appearance of the male and female characters. Since all of the larvae develop

under practically identical conditions, it does not seem likely that these variations can be due to environmental factors.

Many mendelian workers have found it convenient to assume that the appearance of the two sexes in approximately equal numbers in most animals is due to the fact that one sex is heterozygous for sex, while the other is homozygous for this character. This suggestion comes from the fact that the sex ratio corresponds with the ratio obtained when a first-generation hybrid is bred to a pure recessive. In this case half of the offspring will be pure recessives or homozygotic, while the other half will be hybrid or heterozygotic with the dominant character present. From the study of the sex chromosomes it seems to have been found that one sex may produce two kinds of germ cells which are visibly different, while the other sex produces only one kind. It appears further that it is sometimes one sex and sometimes the other that is heterozygous with regard to the sex character. It has been supposed, and is still maintained by a number of investigators, that the sex chromosomes are absolute sex determiners. The idea, however, that the chromosomes act qualitatively has given way to the belief that the influence exercised by the sex chromosome is a quantitative one, and this conception has paved the way for a better understanding of the sex phenomena in forms that exhibit hermaphroditic tendencies. The conception that all individuals carry the factors of the opposite sex in a latent condition will probably prove to be correct, and it may lead to a general acceptance of the theory that sex is not unalterably fixed at the time of fertilization.

The primary difference between a male and a female in any species is not as great as one might conclude from the appearance of the adults of the two sexes. The first sexual changes usually take place in the germ gland; in the female some of the germ cells very early stop dividing and enter upon a period of growth, while in the male the germ cells continue to multiply for a long time.

Since the primary difference between the female and the male is, that in one the germ cells enter early upon a period of growth, while in the other they continue to divide, it seems probable that



one or the other mode of development is the result of a difference in the body metabolism in the two kinds of individuals, and is not due to the inheritance of unalterable sex factors. If sex should prove to be the result of slight differences in metabolism, it would be easy to understand how a reversal of sex might take place under certain circumstances. It must be admitted that these metabolic differences might exist in the animals from the very beginning of development, and, in so far as they are transmitted by the parental germs which unite in fertilization, they may be said to be inherited. If the sex characters are to be compared with other so-called mendelian characters, we have to admit also the possibility of a quantitative variation in the latter, which seems to be contrary to the opinion of the majority of geneticists at the present time.

From evidence already presented we are forced to the conclusion that in many dioecious forms, at least, every individual is a potential hermaphrodite, in so far as it carries the latent qualities of the opposite sex. Whether an animal develop into one or the other sex may depend upon an inheritable quantitative relationship existing between the male and female potentialities in the fertilized egg. As has already been mentioned, there seems to be some evidence that in some forms it is one and in other forms it is the other of the two sex cells that unite in fertilization which is responsible for the quantitative difference of the sex factors. The theory advanced by Castle ('03), that both male and female cells are heterozygous with regard to sex, required the assumption that selective fertilization was necessary in order to bring about the observed results. This has been objected to on account of the lack of evidence that there is such a selection among the germ cells. Papanicolaou ('15) and Stockard and Papanicolaou ('16) have brought forward some evidence that selective fertilization might take place in the case of guinea-pigs, but the full data have not, as far as I know, been published.

If it be found necessary to consider one sex heterozygous for sex and the other homozygous, the formula that appears most applicable is that adopted by Goldschmidt, which has been accepted with some modifications by Witschi and others.

In the case of the lamprey, it does not seem necessary to tie the question of sex up with chromosomal constitution. It is easy to conceive of every fertilized egg as being practically in a balanced condition as regards sex. Some may be more strongly inclined in one direction and some in the other, and, in so far as this is so, the sex characters may be considered as inherited. But if we look upon the development of one or the other sex as a result of metabolic differences, there is no necessary reason why these differences should be referred to the chromosomal make-up of the fertilized eggs. They may equally well be the result of cytoplasmic differences in the eggs, and these may be present even before fertilization. If we consider sex from this standpoint, it is not difficult to understand how, as a matter of chance, there might be an equality of males and females when conditions of development are normal, and also to understand how, under extraordinary circumstances, sex might be altered in the developing organism. A cytoplasmic inheritance of the female characters (FF) has been suggested by Goldschmidt for the gypsy-moth. In the case of *Sagitta*, which is hermaphroditic, it is claimed by Elpatewsky that the development of a primordial germ cell into a male or a female cell is dependent upon the proportion of the cytoplasmic body, the so-called 'besondere Körper,' that each cell receives.

Whether we consider the chromosomes or some other part of the cell as responsible for the determination of sex, we must, in the last analysis, think of sex determination as due to the relation between two opposite potencies which are both present in the fertilized egg. In true hermaphrodites, in which male and female germ cells are matured simultaneously, the two potencies are in a state of equilibrium, so that the presence of one is not antagonistic to the other. In protandric and protogynous hermaphrodites the two sexual states seesaw back and forth so that each alternately replaces the other, while in a case like that of *Crepidula*, which under normal conditions is male in the young stage and female in the older stage, the male potency never reappears. In the two latter cases we may think of the antagonism between the two sexual states as the result of the action of cer-

tain hormones, secreted during the development of one or the other form of sex cell, and which inhibits the development of the other. When one set of sex cells is exhausted, the action of the hormones ceases and the other set of cells begins to develop.

In dioecious animals and plants the two forces do not exert themselves in the same individual except as the result of unusual conditions. In the free-martin, for example, the female potency is in the lead from the beginning of development; but by the action of hormones circulating through the body of the embryo, the male factor asserts itself so that the female factor is partially suppressed, even to the extent "that a gonad with a primary female determination may form a structure which is morphologically a testis" (Lillie, '17, p. 468). When the spider-crab, *Inachus*, is infected with the parasite *Sacculina*, the males, parasitically castrated, may show every degree of modification toward the female state, even to the appearance of ova in the remaining part of the testis. The females, however, are not transformed toward the male condition, and the conclusion is drawn by Geoffrey Smith that the male is heterozygous for sex and the female homozygous. Such a conclusion is hardly warranted, for the parasite does not seem to act simply by arresting the action of one sex potency, but by also elaborating certain materials which are favorable to female development. That this is so may be surmised from the fact that when immature females are infected, the effect is "to force them to assume prematurely adult female characteristics" (Smith, '10). There is no reason, therefore, why a female, when infected, should be transformed toward the male side.

In pigeons, sex seems to be a matter of metabolic difference, and a disturbance of the metabolic level may be brought about by hybridization as well as by overwork in reproduction so that a sex reversal is effected. In this case Riddle thinks he has demonstrated "that germs *normally* female-producing, have, under experiment, been made to develop males; and that germs which were prospectively male-producing have been made to form female adults" (Riddle '16, p. 410). In the case of the gypsy-moth, hybridization again seems to disturb the sex metabolism

so that a prospective female develops male characters and a prospective male develops female characters. Whether or not complete reversal of sex has occurred in moths, does not appear from literature on the subject, although there are apparently cultures yielding nothing but males (Goldschmidt, '17, p. 605).

In *Bonnelia* the sex metabolism is disturbed by environmental conditions. This is also true in the case of *Crepidula*. In frogs, delayed fertilization determines the results, as possibly also does temperature to a certain extent. In rotifers the change may be the result of the amount of oxygen present in the culture (Shull and Ladoff, '16; Shull, '18). Whitney, however, has obtained different results with rotifers. From the various cases of sex reversal in plants, mentioned above, it appears that in these cases the change is effected by disturbing in one way or another the normal conditions under which the plant lives.

It seems to be amply proved that among dioecious animals and plants every individual carries the qualities of the opposite sex in a latent condition. This is a great step toward the solution of the problem of sex determination; but it remains to explain why in some cases one potency asserts itself, while in other cases the other appears. Opinions on this question converge around the conception of a variableness in cell metabolism and the action of enzymes. Riddle thinks of male- and female-producing eggs, in the case of pigeons, as different in regard to their storage capacity—a less storage capacity pertains to the male—and a high storage capacity pertains to the female-producing germ. Riddle says: "The progressive *increase* in storage capacity of the eggs during the season—under overwork—is to be interpreted as a *decrease* in the oxidizing capacity of the same eggs." This opinion is similar to that expressed by Shull in the case of rotifers.

The metabolic capacity of the germ is, of course, reflected in the adults derived from them: We can easily see how, in forms like the lamprey, the storage capacity and the oxidizing capacity may so nearly balance each other that every larva may exhibit both tendencies in different parts of the body. This, as has been suggested above, may be due to slight inequalities in the cells resulting from division or to environmental factors of some sort

influencing the germ cells in different parts of the gonad. Thus we may have, in the same gonad, certain cells with high oxidizing capacity which continue to divide and form cell nests, and other cells, with a high storage capacity, which enter the growth period very early and become oocytes. After the larva has become decidedly male or female in character, as evidenced by the proportion of cell nests and oocytes, it appears that the opposite sex tendency is in decline. This can be attributed to the presence of sex-differentiating enzymes produced by the predominating sex character. This results in the arrest of the development of the opposite sex character and often in its degeneracy.

Goldschmidt has worked out a theory of enzyme action in connection with sex which seems to be in the right direction. He assumes that "in the fertilized egg the enzymes which govern the differentiation of the organism towards one of the two alternatives, maleness and femaleness, are both present." These hypothetical enzymes he speaks of as andrase and gynase. The distribution of the sex chromosome "results in the formation of two kinds of fertilized ova, differing in the relative concentration of the two enzymes." Since in "mixtures of different enzymes, every single one reacts independently, providing no interfering reaction product is formed," a decision must be reached during differentiation of the organs as to whether they shall develop along the male or the female line. "This decision must be brought about by the action of the dominating enzyme." The more nearly the two enzymes approach each other in strength, the earlier do they show their double influence on the developing organism. Such seems to be the case in the intersexual moths which show all degrees of intersexuality, from slight changes in the secondary sexual characters, which are latest to be formed, to changes in the germ gland itself, which is the first sex organ to differentiate. The same idea may be applied to the lamprey, where various grades of intersexuality are found in the germ gland, and where the sex differentiating factor seems to operate early in some of the larvae and later in others.

We are not, strictly speaking, concerned in the present work with the causes which underlie the development of the secondary

sexual characters or the accessory reproductive organs, since there are none in the larval stages of the lamprey. In insects the secondary sex characters appear to be unaffected by the presence or absence of the sex glands, but in most other forms the appearance of the secondary sexual characters may be the result of certain hormones which are produced by the predominating primary sex elements. This appears to be the case in the lamprey. Sooner or later in the life of the individual the male- or the female-producing enzyme, if such it be, takes the lead so that the action of the opposite enzyme is more and more suppressed. The male or the female germ gland, which develops as a consequence of the stronger enzyme, is capable of producing certain hormones which, both at and after metamorphosis, cause the secondary characters to appear.

The above conception is not opposed to the theory that the so-called sex chromosomes are associated with the phenomena of sex in many cases. The evidence indicates, however, that they are only one link in a series of processes which result in sex determination, and that other factors may operate so as to change development, in spite of the presence of the sex chromosome. The physiological action of the sex chromosome may be fundamentally the same as that of other factors.

Finally, if we think of sex as an hereditary character, as it seems we must, then it is amply demonstrated that here we have an hereditary character that can be modified by a variety of circumstances. Unless we assign the sex character to another category than other hereditary characters, we are forced to acknowledge the possibility that other hereditary characters are modifiable also. If this should prove true, it is possible that the idea that the sex character is changeable will be accepted with less reserve than heretofore.

## GENERAL SUMMARY OF OBSERVATIONS

*A. Origin and early history of the germ cells*

1. The germ cells are first recognizable in the American brook lamprey when the mesoderm separates from the entoderm, as large yolk-laden cells which become included in the mesoderm. Their history previous to this time could not be traced. Their large size, however, indicates that they are early segregated cells.

2. The number of germ cells that become included in the mesoderm is small. There is evidence that many of them never reach the germ-gland region. Some of these degenerate before dividing, others form cysts in other regions of the body, and the possibility is suggested that some of them may be extruded into the lumen of the intestine in early stages.

3. During the early period of their history the germ cells shift from a lateral position in the mesoderm to a median position. The change in position is accredited to a shifting of the tissues surrounding the cells and, to a lesser extent, to independent migration.

4. The germ cells begin to lose their yolk when the larva is about 5.5 mm. long, and no yolk remains in the cells when the larva is 10 mm. long. They do not begin to divide until the larva is about 20 mm. long.

5. The germ cells may be distinguished from the somatic cells by their size, structure, and location.

*B. Period of secondary division*

6. When the larva is about 20 mm. long the germ cells begin to divide by mitosis.

7. After each mitosis the germ cells either separate or remain together, forming cell nests. Peritoneal cells migrate in and form follicles around the individual cells and cysts.

8. An astrosphere is distinguishable in the germ cells of this stage.

9. A vitelline body is found in the cytoplasm. Its origin could not be ascertained. It becomes a very prominent structure in the growing oocyte of later stages.

10. Numerous mitochondria are present in the cytoplasm of the germ cells in most phases of their history.

11. Two plasmosomes are present in the primordial germ cells but in the growing oocyte there is only one.

12. The period of secondary division lasts until the larva is about 35 mm. long. During this period the larva appears indifferent as to sex.

### *C. Period of sex differentiation*

13. The period of sex differentiation extends from the time the larvae are about 35 mm. in length until they are about 70 mm. long. In some larvae, however, sex differentiates much earlier than in others. During this period the sex of the larvae is indeterminate. The condition may be described as juvenile hermaphroditism.

14. A varying number of oocytes appear in practically all the glands during this period, so that a series of glands might be arranged possessing from 0 per cent to 100 per cent of oocytes.

15. The changes taking place in the oocytes during the synapsis phase are described.

16. Numerous germ cells degenerate during this period. Degeneration may take place during the synapsis phase, the growth phase, or the indifferent phase of the germ-cell history.

17. When sex is established the germ cells belonging to the opposite sex disappear or remain in the gland in a rudimentary condition.

### CONCLUSIONS

The following general conclusions may be drawn from the above study of the germ cells of the American brook lamprey:

1. The germ cells are segregated very early in the life of the animal even before the germ layers are definitely established. They are first recognizable when the mesoderm separates from the entoderm.

2. The definitive germ cells take their origin from no other source than the primordial germ cells and the germ cells take no



part in the formation of somatic structures. Numerous germ cells are produced which do not become functional, and these degenerate and disappear during the process of development.

3. The germ cells of each germ gland are usually of two kinds namely, those showing a tendency toward rapid division (katabolic) and those showing a tendency for growth (anabolic). The former are regarded as having a male, the latter a female potentiality. The relative proportion of anabolic and katabolic cells determines whether the larva becomes a male or a female individual.

4. Observations seem to warrant the conclusion that each larva of this species carries the potentiality of both sexes, and that sex, therefore, is not irrevocably fixed at fertilization.

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## PLATES

## ABBREVIATIONS

<i>arc.</i> , archenteron	<i>gon.</i> , gonad
<i>ast.</i> , astrosphere	<i>i.e.</i> , intestinal epithelium
<i>bv.</i> , blood-vessel	<i>lu.i.</i> , lumen of intestine
<i>c.</i> , centrosome	<i>m.</i> , mitochondria
<i>ch.</i> , chromidia	<i>m.b.</i> , midbody
<i>co.</i> , coelom	<i>m.c.</i> , mesenchyme cell
<i>cy.</i> , cyst	<i>mes.</i> , mesoderm
<i>d.a.</i> , dorsal aorta	<i>mesn.</i> , mesonephros
<i>deg.cy.1.</i> , degenerating cyst, early stage	<i>m.f.c.</i> , migrating follicle cells
<i>deg.cy.2.</i> , degenerating cyst, late stage	<i>n.</i> , nucleus
<i>d.g.</i> , dividing germ cell	<i>nch.</i> , notochord
<i>deg.g.</i> , degenerating germ cell, early stage	<i>n.t.</i> , nerve tube
<i>e.c.</i> , extruded germ cell	<i>o.</i> , oocyte
<i>ex.cy.</i> , extraregionary cyst	<i>p.c.v.</i> , postcardinal vein
<i>f.c.</i> , follicle cell	<i>pr.d.</i> , pronephric duct
<i>g.</i> , germ cell	<i>st.</i> , stroma
<i>g.c.r.</i> , germ cell region	<i>t.</i> , tetrad
<i>g.e.</i> , germinal epithelium	<i>v.</i> , vitelline body
<i>g.f.</i> , germ cell fragment	<i>w.f.</i> , wolffian duct
	<i>y.</i> , yolk

All outlines were made with a camera lucida.

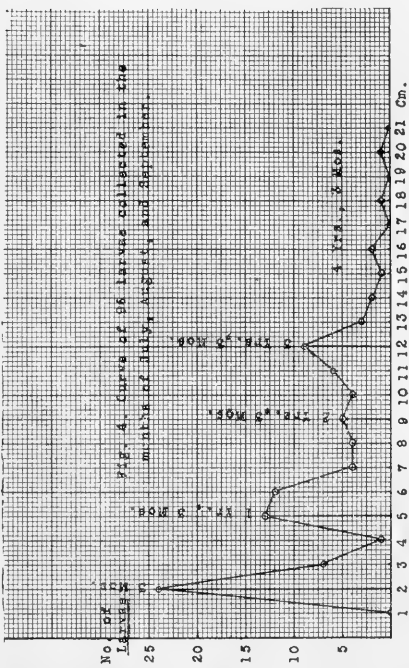
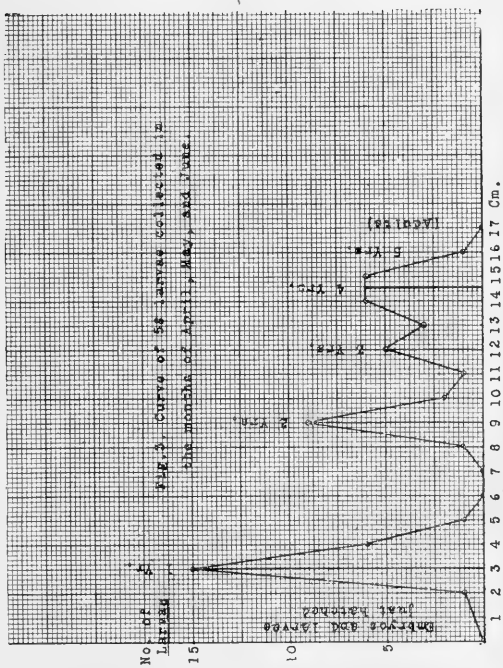
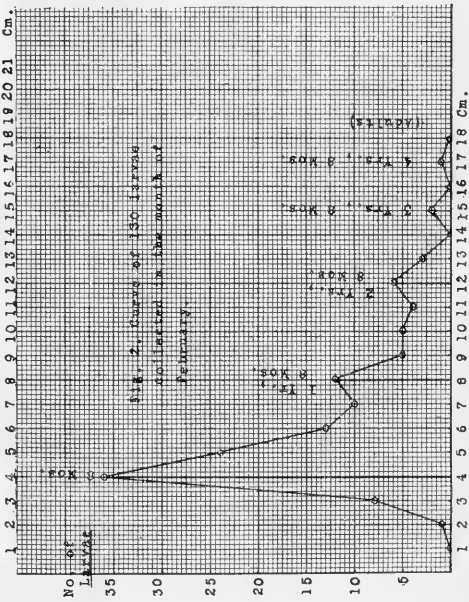
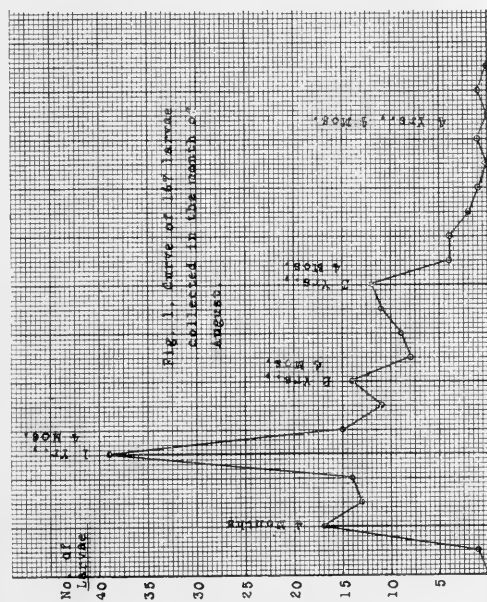
## PLATE 1

### EXPLANATION OF FIGURES

1 to 4 Curves showing frequency distribution of the length of larvae of *Entosphenus wilderi* collected during various months of the year. The ordinates represent numbers of individuals, the abscissae lengths in centimeters.

GERM-CELL HISTORY IN THE BROOK LAMPREY

PETER OKSBERG



## PLATE 2

### EXPLANATION OF FIGURES

5 Embryo of *Entosphenus wilderi*, 191 hours old. The black area represents the germ-cell region. The line *ab* is the plane of the section in figure 11.

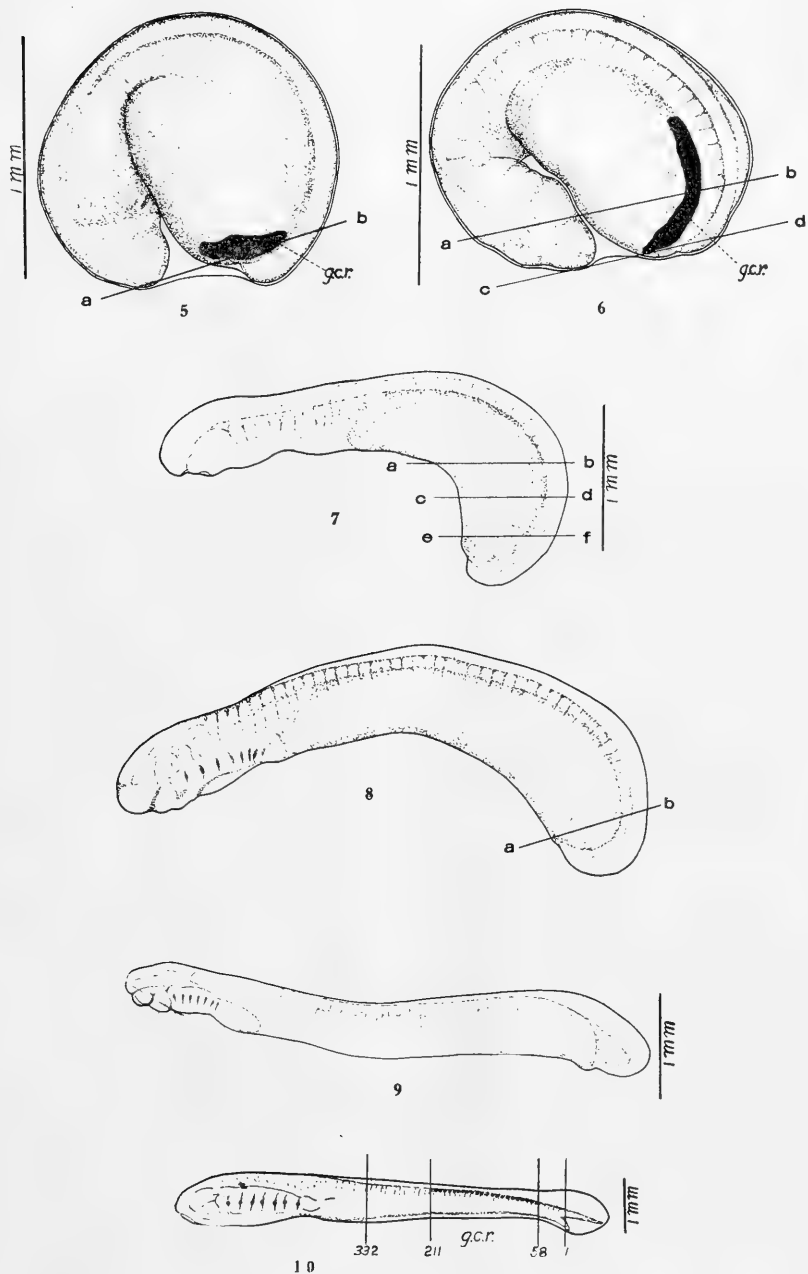
6 Embryo 238 hours old. The shaded area indicates approximately the position of the germ cells. The planes *ab* and *cd* are the planes of the sections in figures 12 and 13.

7 Larva 274 hours old. The lines *ef*, *cd*, and *ab* are the planes of the sections in figures 14, 15 and 16, respectively.

8 Larva 299.5 hours old. The line *ab* is the plane of the section in figure 17.

9 Larva 359.5 hours old.

10 Larva 8 mm. long (37 days, 14.5 hours). The distribution of the germ cells is shown by dots. Thirty-six germ cells were found in a larva of this stage. The position of the various  $10\mu$  sections craniad of the anal aperture is shown by vertical lines.

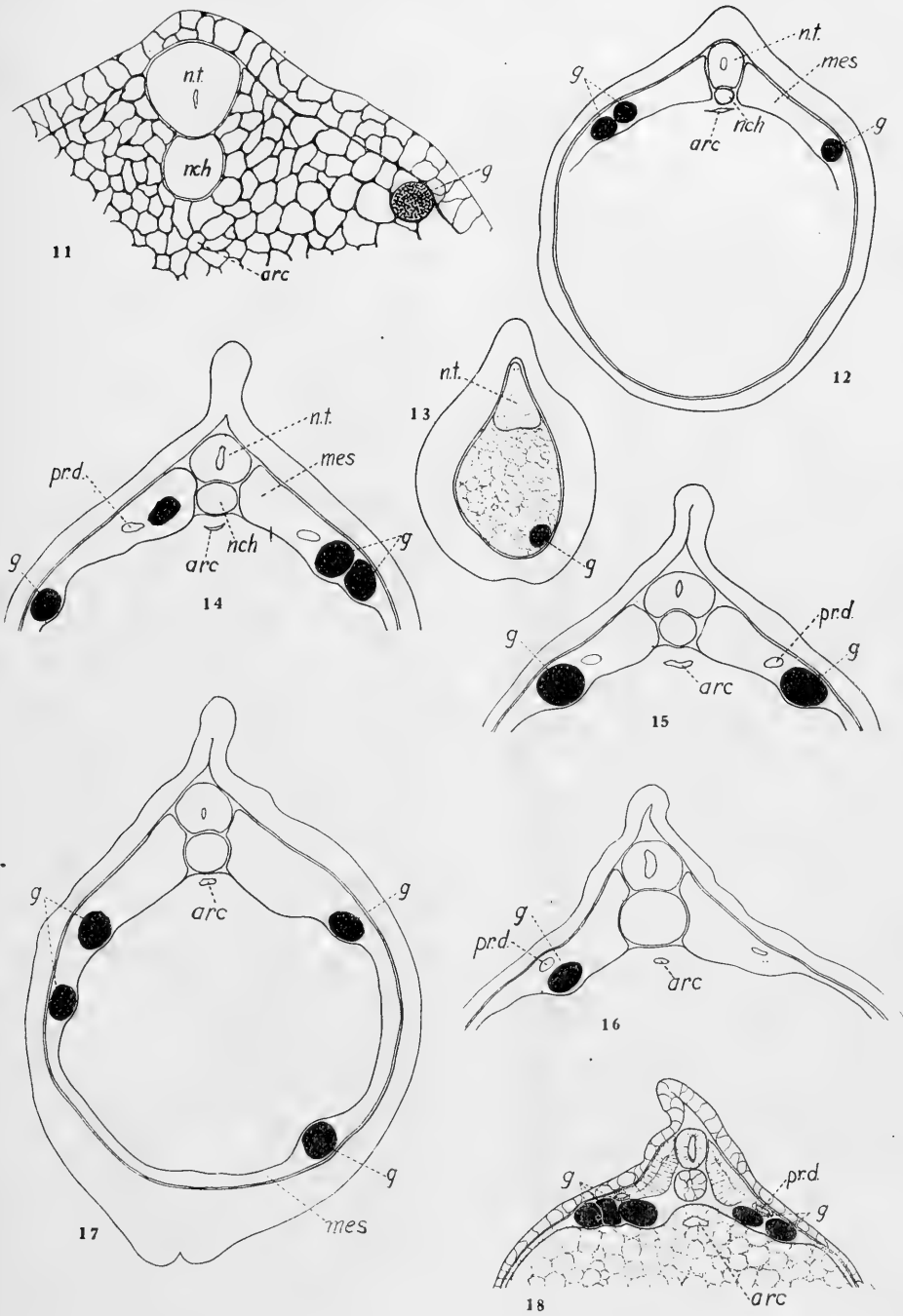


### PLATE 3

#### EXPLANATION OF FIGURES

- 11 Section through the caudal region of the embryo shown in figure 5.
- 12 and 13 Sections of the embryo shown in figure 6 through the regions *ab* and *cd*, respectively.
- 14, 15, and 16 Sections of the larva shown in figure 7 through the regions *ef*, *cd*, and *ab*, respectively.
- 17 Section of the larva shown in figure 8 through the region *ab*.
- 18 Section of a larva 286.5 hours old.





## PLATE 4

### EXPLANATION OF FIGURES

19 Section through the middle of the body of a second larva 299.5 hours old (figs. 8 and 17).

20 Detail structure of germ cells from a larva 359.5 hours old. One germ cell is cut through the nucleus, the other along one side.

21 Section of a larva 373.5 hours old. The coelom is forming at this stage on each side of the intestine. One germ cell is shown near the ventral mesentery.

22 Section through the middle of the body of a larva 429.5 hours old.

23 Section through the caudal region of a larva 429.5 hours old (same larva as the preceding figure). The coelom is forming and a germ cell (*g*) is included in the somatic layer of the mesoderm.

24 Section through the middle part of the body of a larva 478.5 hours old. The germ cell in the figure is at the cranial end of the germ-gland anlage.

25 The germ cell in figure 26 greatly enlarged. It shows disintegration of yolk globules and the elimination of chromatin-like material from the nucleus.

26 Section through the middle of the body of a larva 538.5 hours old. The yolk is beginning to disappear in the most cranial germ cells.

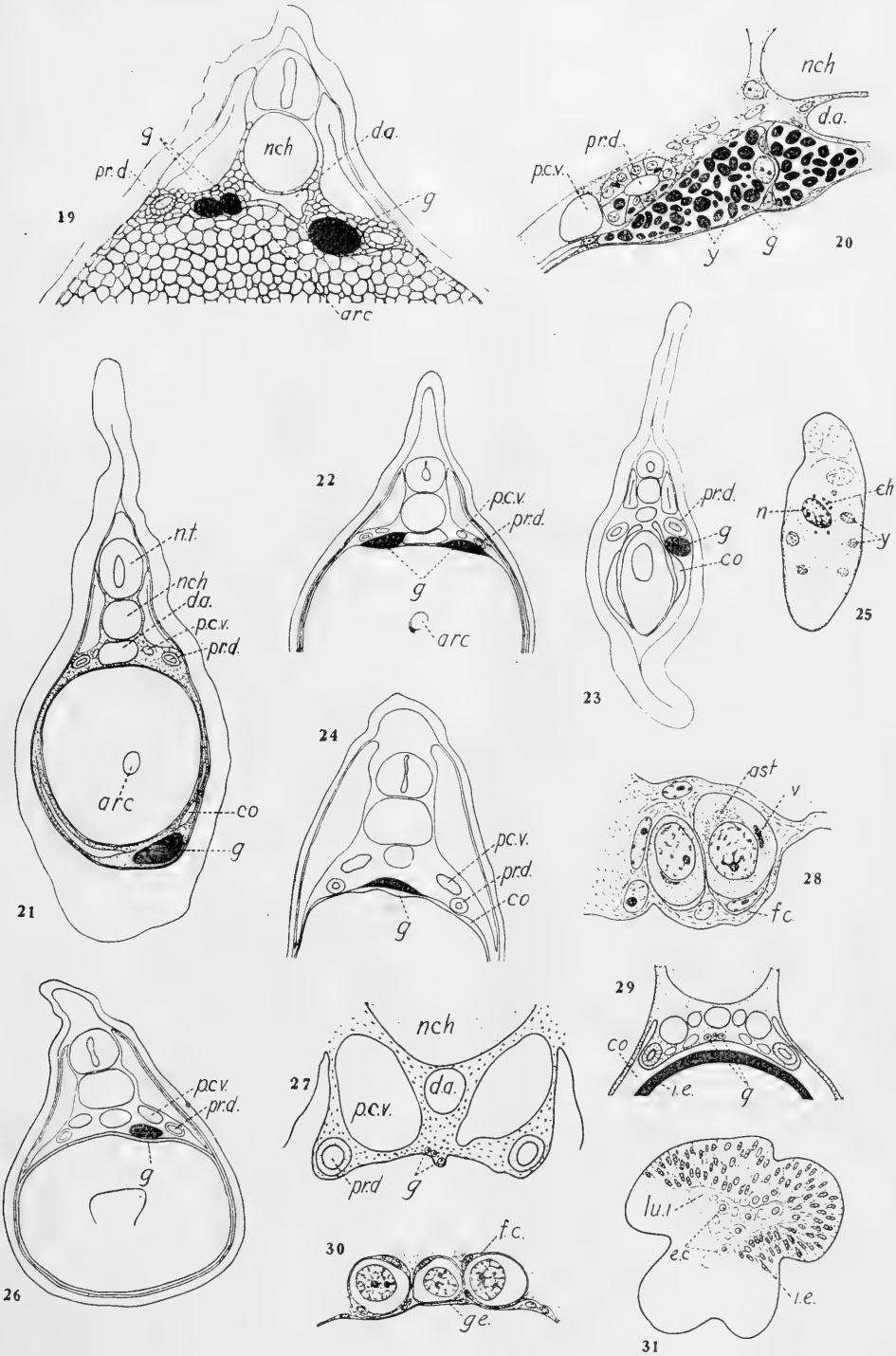
27 Section through the germ-gland region of a larva 20 mm. long. The germ ridge is forming and the germ cells are migrating into it.

28 Enlarged drawing of two germ cells from the same larva as the preceding figure, showing astrospheres and vitelline bodies.

29 Section of a larva 11 mm. long in the region of the germ gland. At this stage the germ cells have lost their yolk.

30 Enlarged drawing of the germ cells shown in figure 29. The germ cells lie above the germinal epithelium and are not a part of it. There is no germ ridge at this stage.

31 A section of the intestine near its caudal end from a 10-mm. larva. The lumen of the intestine is filled with cells that have been extruded.



## PLATE 5

### EXPLANATION OF FIGURES

32 Section through the cranial end of the coelom of a larva 35 mm. long, showing a nest of germ cells in the fat-body above the mesonephros.

33 Section through the germ-gland region of a larva  $4\frac{3}{8}$  cm. long, showing nests of germ cells in different parts of the mesonephros.

34 Section of the germ gland of a larva 21.5 mm. long.

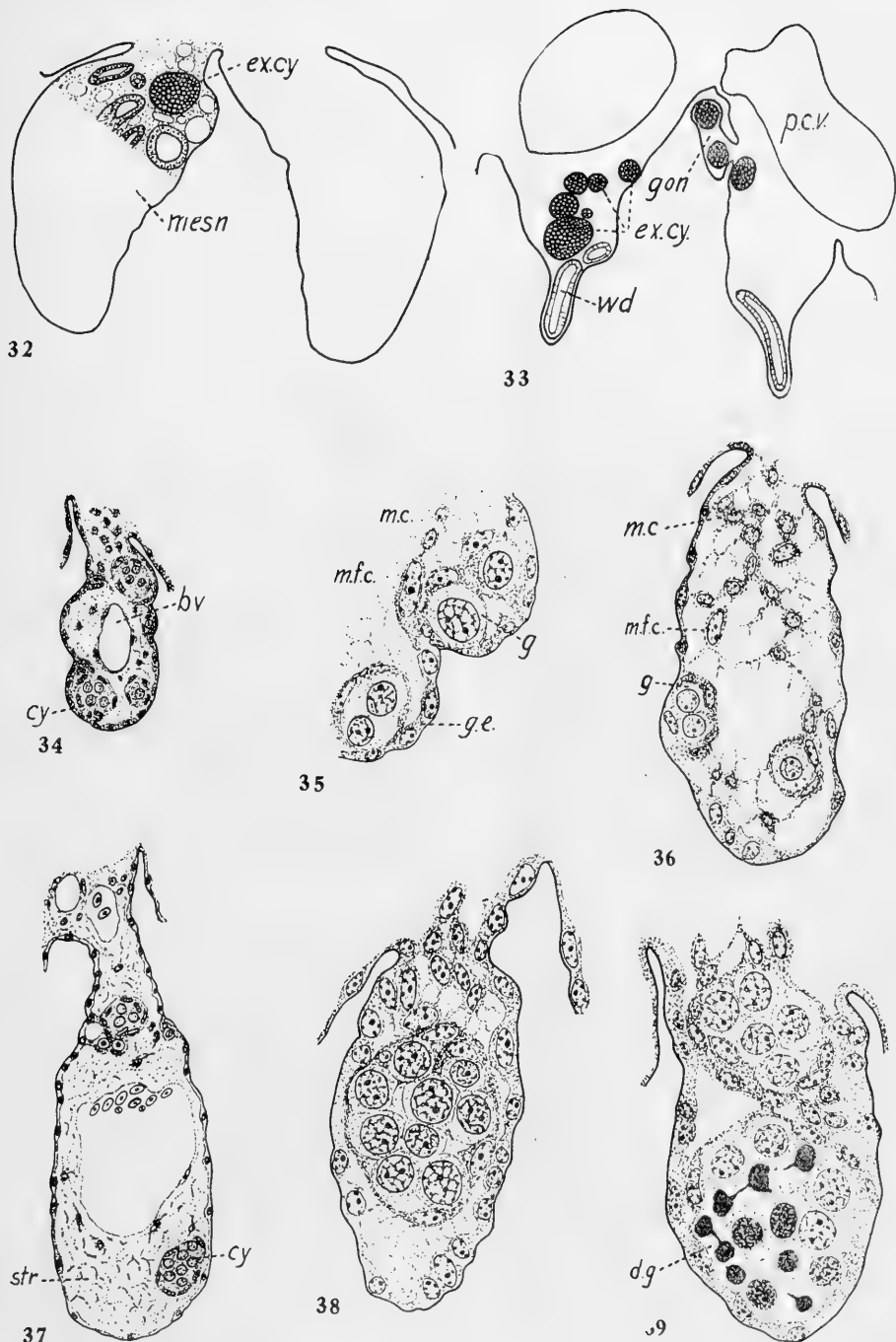
35 A magnified portion of the germ gland of a larva 25 mm. long, showing the inward migration of peritoneal cells to form follicles around the germ cells.

36 Section of the germ gland of a larva 21.5 mm. long, showing scattered germ cells and mesenchyme cells.

37 Section of the germ gland of a larva 25 mm. long.

38 Section of the germ gland of a larva 27.5 mm. long, showing a cyst of germ cells surrounded by follicle cells. The outlines of the individual germ cells are represented.

39 Section through the same gland as in figure 38 showing a cyst with dividing cells. Some of the germ cells of the cyst are in a resting stage.



## PLATE 6

### EXPLANATION OF FIGURES

40 Portion of a germ gland of a larva 30 mm. long, showing the breaking up of a cyst by the inward migration of follicle cells.

41 A resting germ cell from a larva 42.5 mm. long. The gland was fixed in Meves' solution and stained in iron hematoxylin. Mitochondria are abundant in the cytoplasm.

42 A germ cell in early prophase from a larva 30 mm. long.

43 A germ cell in prophase somewhat more advanced than that in figure 42.

44 Germ cell in middle prophase from a larva 47.5 mm. long. The chromatin network has broken up and the individual chromosomes are free. Only one nucleolus is present.

45 Side view of a cell in which the chromosomes are arranged on the equatorial plate.

46 End view of a cell in the same stage as figure 45. The individual chromosomes are still visible.

47 Early anaphase in which a chromosome-like body lies outside of the spindle.

48 Early anaphase in which the chromosomes are migrating toward the poles. Some of the chromosomes begin their migration earlier than others.

49 A germ cell in late anaphase from a larva 27.5 mm. long.

50 A germ cell in late anaphase showing a distinct midbody.

51 Telophase showing reconstruction of the nuclei. One nucleolus has made its appearance in each cell.

52 An oocyte in the early synaptic phase, from a larva 77.5 mm. long. Mitochondria are numerous at this stage.

53 Early leptotene stage from the same larva as the preceding figure. Both astrosphere and vitelline body are present.

54 Advanced leptotene stage from the same larva as the preceding figure.

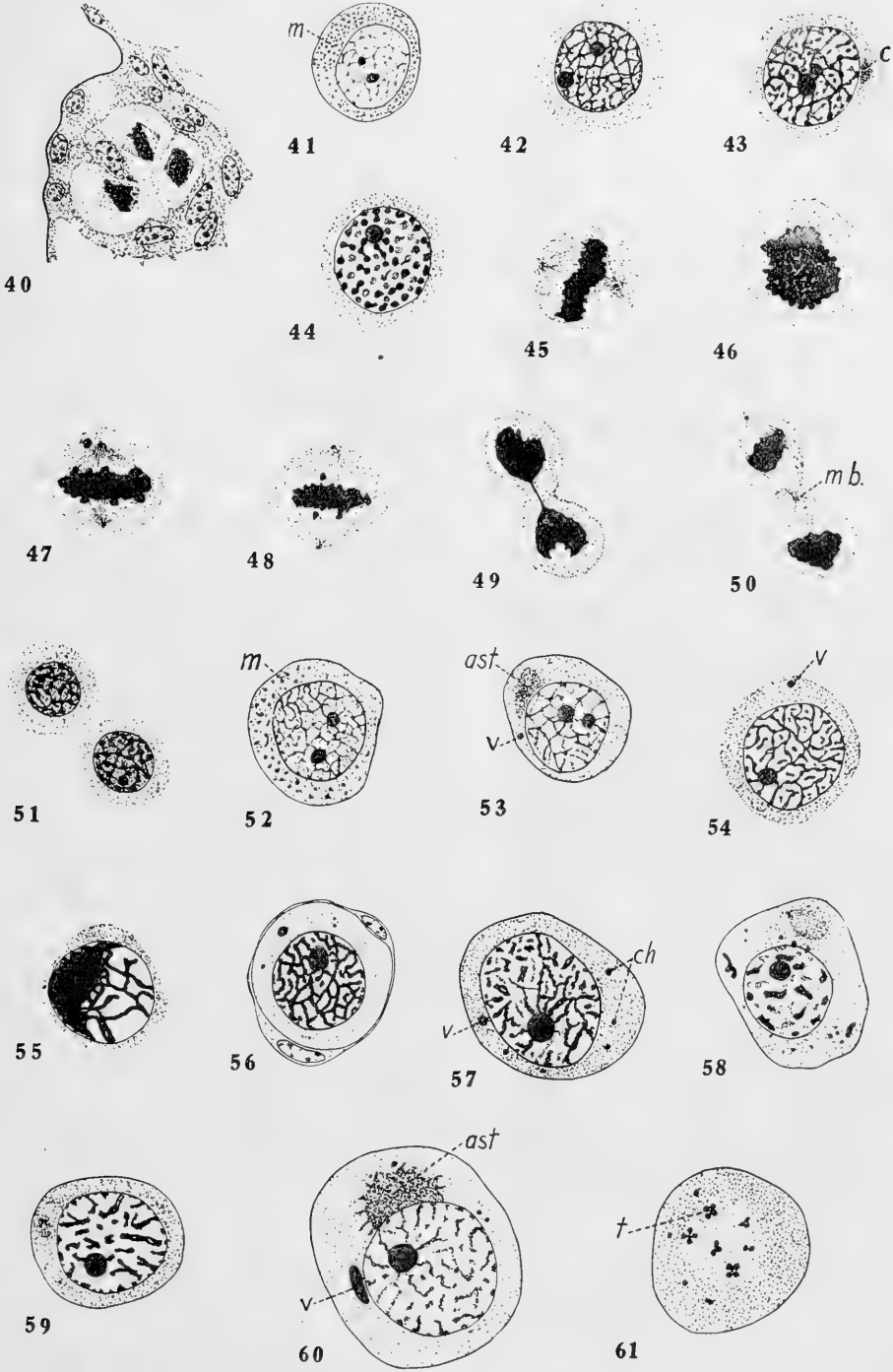
55 Synaptene stage (bouquet stage, synizesis stage), from a larva 59 mm. long.

56 Late pachytene stage from the same larva as the preceding figure.

57, 58, and 59 Various phases of the diplotene-diacyate stage. The chromosomes are paired; chromatin-like bodies (chromidia) are found in the cytoplasm; only one nucleolus is present.

60 An oocyte in the early growth phase. The vitelline body is a prominent structure at this stage.

61 A growing oocyte sectioned through the surface of the nucleus, showing the arrangement of the chromosomes in tetrads.



## PLATE 7

### EXPLANATION OF FIGURES

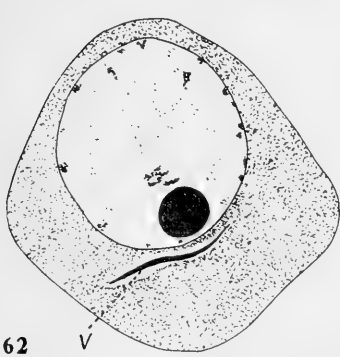
62 An oocyte in the early growth phase, showing the vitelline body in the form of a spindle.

63 Section through an adult testis, showing an oocyte among the numerous cysts (not filled in).

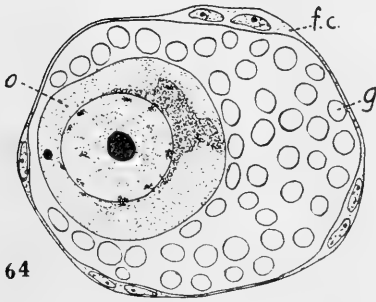
64 Section through a cell nest containing one growing oocyte among numerous smaller germ cells.

65 Section of a degenerating cyst, with germ cells in various stages of disintegration.

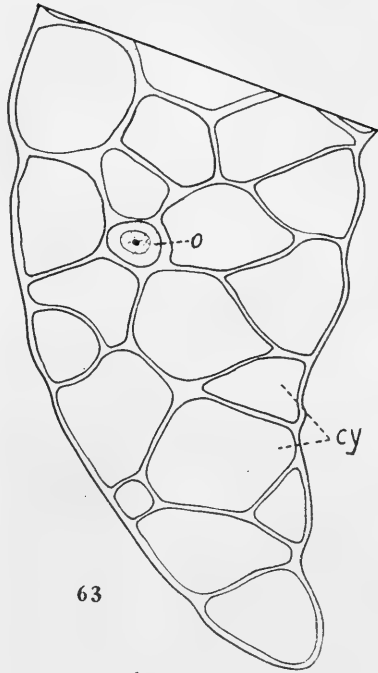




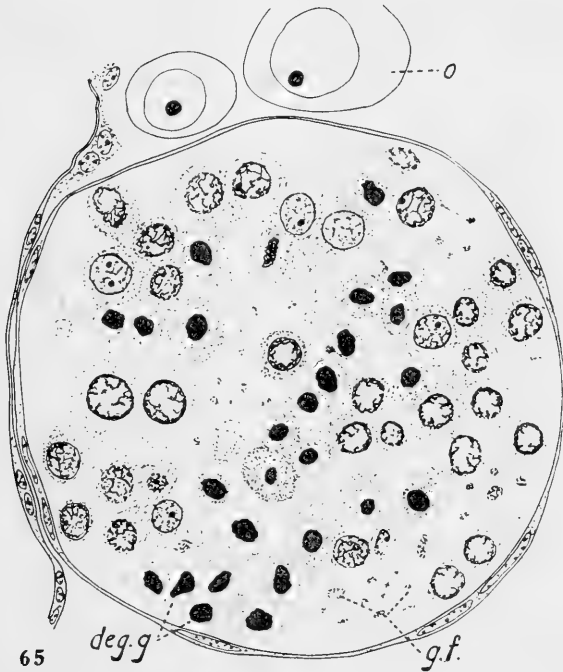
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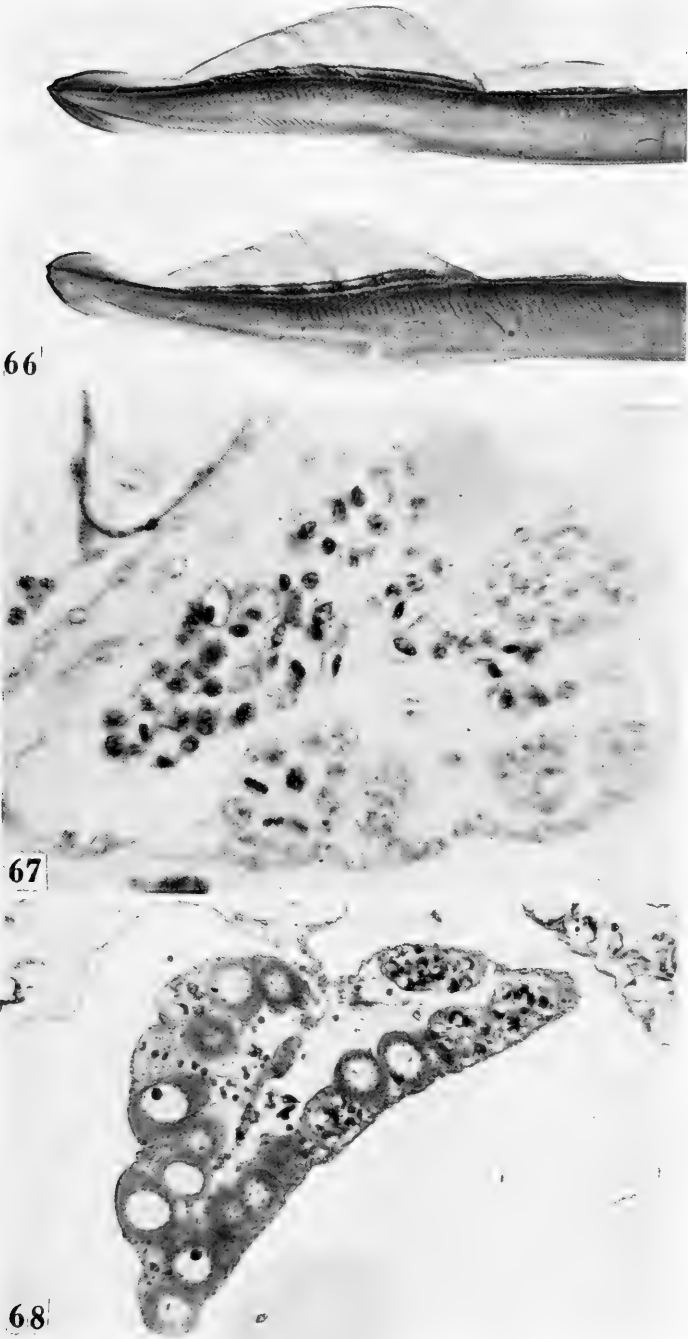
## PLATE 8

### EXPLANATION OF FIGURES

66 Photograph of the caudal regions of a male and a female brook lamprey, showing the external sex characters.

67 Microphotograph of a germ gland of a larva 47.5 mm. long, showing a cell nest with dividing and resting cells.

68 Section of the germ gland of a larva 54 mm. long, showing a cyst with dividing cells on the left, cysts with cells in synizesis on the right, and several growing oocytes.



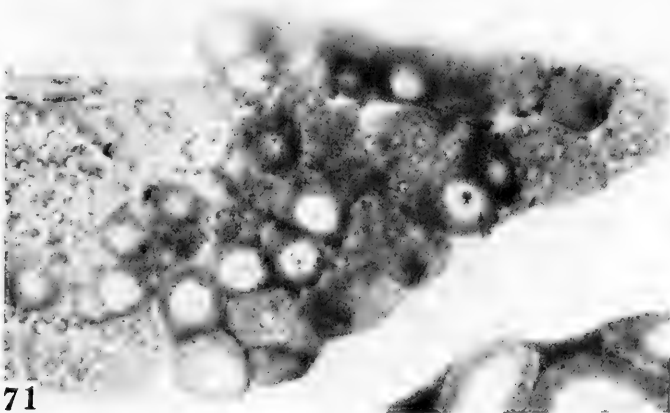
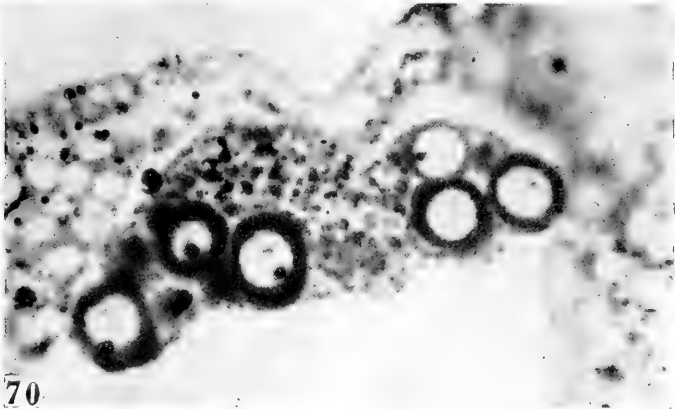
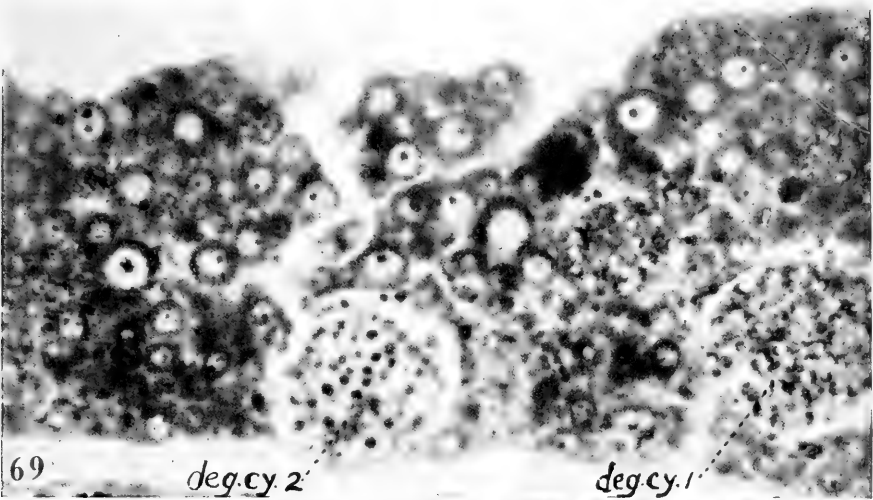
## PLATE 9

### EXPLANATION OF FIGURES

69 Longitudinal section of the germ gland of a larva 59 mm. long, showing two cell nests in different stages of degeneration.

70 Section of the germ gland of a larva 55 mm. long, showing cell nests and growing oocytes in about equal number.

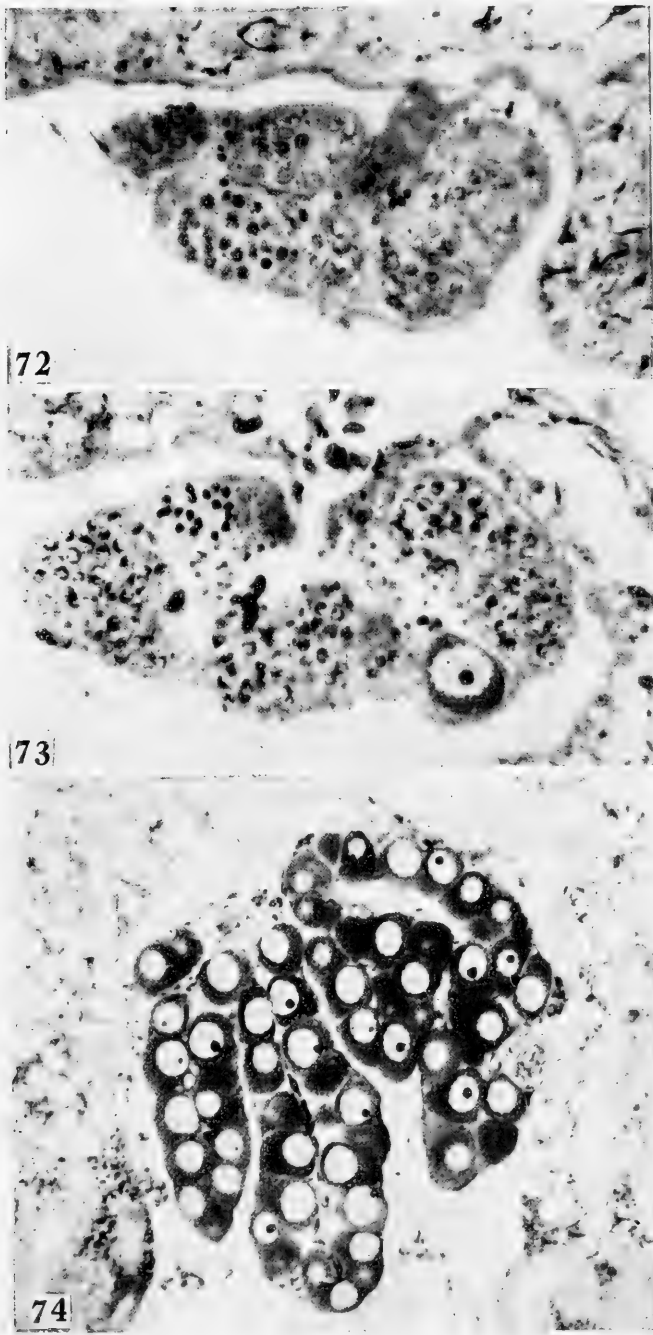
71 Section of the germ gland of a larva 62 mm. long, containing oocytes and cell nests in about equal number.



## PLATE 10

### EXPLANATION OF FIGURES

- 72 Section of the germ gland of a larva 50 mm. long, showing only cell nests.
- 73 Section of the germ gland of a larva 71 mm. long, showing only one growing oocyte.
- 74 Section of the germ gland of a larva 65 mm. long, showing very few cell nests, but numerous growing oocytes.



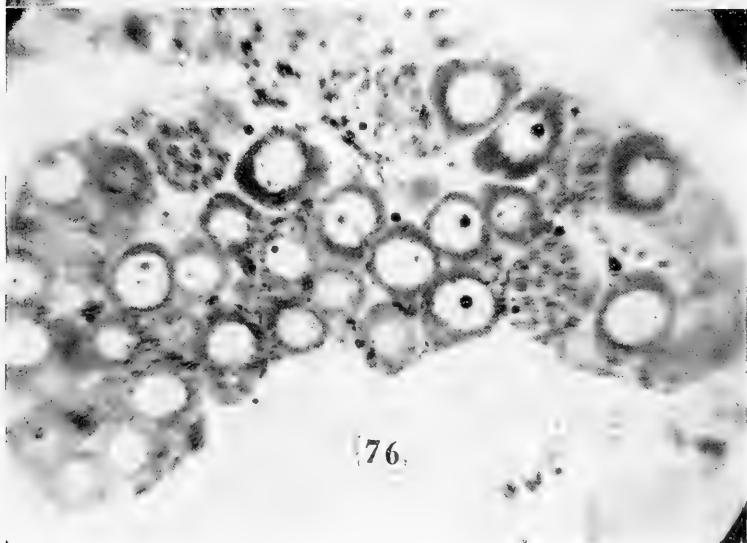
## PLATE 11

### EXPLANATION OF FIGURES

75 Section of the germ gland of a larva 63 mm. long, showing only growing oocytes.

76 Section of the germ gland of a larva 50 mm. long, showing more growing oocytes than cell nests.

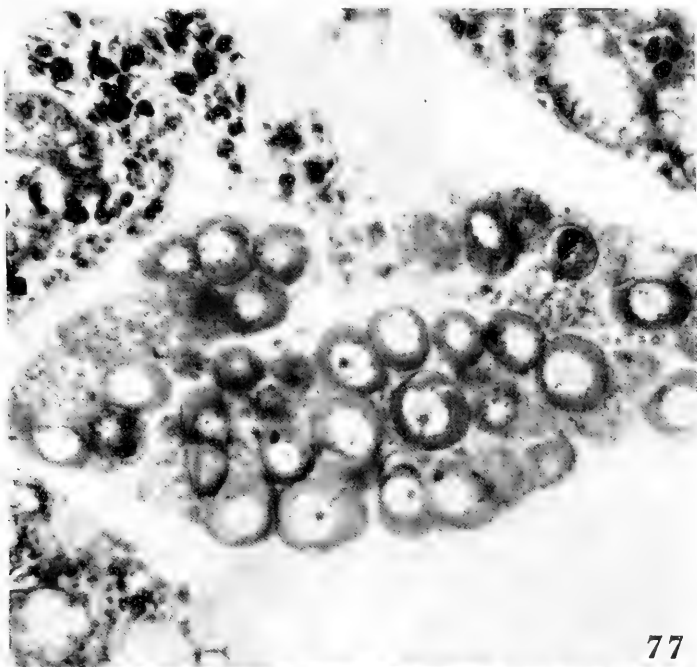




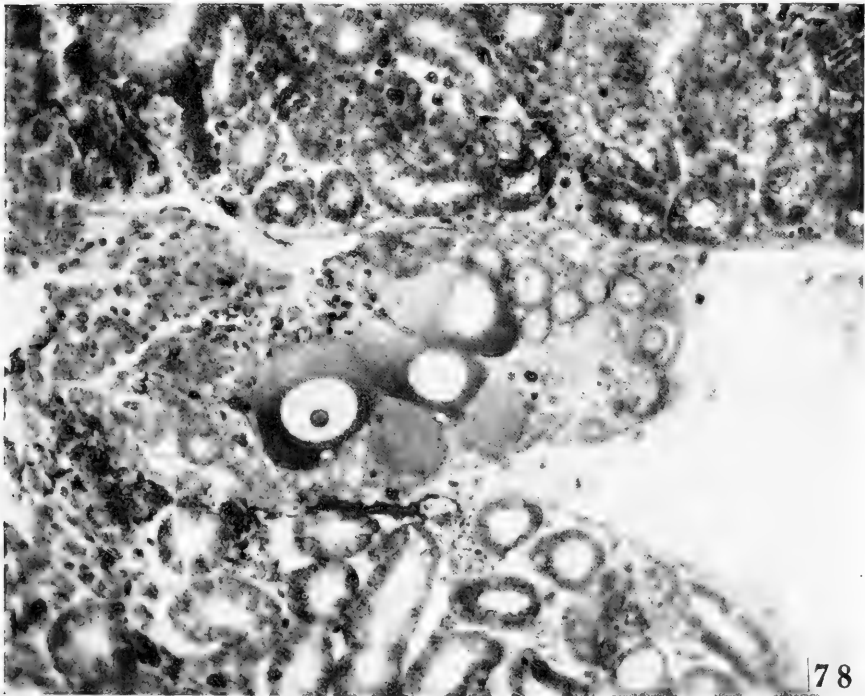
## PLATE 12

### EXPLANATION OF FIGURES

- 77 Section of a larva 60 mm. long, showing the same structure as figure 76.  
78 Section of the germ gland of a larva 72.5 mm. long in which oocytes of various sizes are found.



77



78

Resumen por el autor, Rokusaburo Kudo.  
Universidad de Illinois.

Estudios sobre los Microsporidios, con especial mención de los  
parásitos en los mosquitos.

El autor describe los resultados de un estudio sobre tres Microsporidios de Norte América. *Thelohania magna* Kudo: Esquizogonia por fisión binaria o división múltiple. El cambio nuclear es más complicado que el de *Nosema bombycis*. No se ha podido reconocer fusión de los gametos o unión de dos núcleos al final de la esquizogonia. Un esporonto se transforma generalmente en ocho esporoblastos y a veces en cuatro. El núcleo del esporoblasto se divide en dos núcleos hijos desiguales; uno, más pequeño y situado cerca de un extremo del esporoblasto, es el núcleo del esporoplasma en vías de desarrollo, mientras que el otro, más grande y vesicular está situado cerca del centro del esporoblasto. Este último núcleo produce el filamento polar. Durante estos cambios aparece un espacio estrecho que extendiéndose longitudinalmente se ensancha al avanzar la formación del filamento polar.

*Thelohania illinoisensis* nov. spec. El autor describe las esporas, discutiendo el hecho de que el filamento polar de una misma especie de microsporidio no presenta la misma longitud en todos los casos, dependiendo esto del método empleado.

*Nosema baetis* nov. spec. Esquizogonia por fisión binaria. La espora parece estar organizada de un modo semejante a la de *Nosema bombycis*. El autor discute la diversidad más o menos marcada de la estructura de las esporas de los microsporidios. Por lo menos existen dos tipos que pueden reunirse en un solo grupo; uno de ellos está representado por *Nosema bombycis* y el otro por *Thelohania magna*. El efecto de una infección severa de estos parásitos sobre el animal que los transporta es fatal en el caso de *Thelohania magna*, reduciendo marcadamente la actividad de aquél en el caso de *Nosema baetis*. El autor considera la importancia económica de los Microsporidios en los preliminares del trabajo.

# STUDIES ON MICROSPORIDIA, WITH SPECIAL REFERENCE TO THOSE PARASITIC IN MOSQUITOES<sup>1</sup>

R. KUDO

ONE TEXT FIGURE AND FIVE PLATES (ONE HUNDRED SEVENTEEN FIGURES)

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## INTRODUCTION

In a short note, the writer ('20) reported his observations on the structure of the spore of a new microsporidian parasite, *Thelohania magna* Kudo, from the larvae of *Culex pipiens* collected in the vicinity of Urbana, Illinois. The present paper deals with further observations on the same parasite and on two other Microsporidia found in other aquatic insects from the same locality. The study is fragmentary and the results seem to be far from complete. Yet two of these interesting parasites attack the larvae of two species of mosquitoes and appear to play a more or less important rôle upon the larval existence of these harmful insects. The results of observations up to date have been summarized, and are presented here to call the attention not only of the investigators of Microsporidia, but also of those who may be interested in economic entomology.

<sup>1</sup> Contributions from the Zoological Laboratory of the University of Illinois, no. 169.

## HISTORICAL REVIEW OF THE MICROSPORIDIAN PARASITES OF MOSQUITOES

Careful examination of literature reveals that there have been four Microsporidia recorded as parasitic in this group of dipterous insects, concerning three of which it is doubtful whether they are really Microsporidia or not.

The first record was Pfeiffer ('95) who merely mentioned the occurrence of a 'Glugea' parasite in the larvae of *Culex* sp. As no further description is given, its microsporidian nature is doubtful.

A parasite of the larvae and adults of *Aedes calopus* (= *Stegomyia fasciata*) from Rio de Janeiro was thought by its discoverers, Marchoux, Salimbeni, and Simond ('03) to be a Microsporidian, and was recorded as *Nosema stegomyae* by these authors. Auerbach ('10) replaced this form into the genus *Glugea* on the ground of its polysporogenous character. In their splendid work on mosquitoes, Howard, Dyer, and Knox ('12) mentioned this organism as the only microsporidian parasites of mosquitoes without mentioning *Thelohania legeri* Hesse, which is quoted below. The position of *Glugea stegomyae* in the order Microsporidia, is very doubtful, when one takes into consideration the facts that the authors did not observe the polar filament, which is one of the important characters of the order, in the so-called spores which are from 3 to 7 $\mu$  long and 2 to 3 $\mu$  wide, and that the development, as described by the authors, is extremely different from that of the majority of Microsporidia.

Hesse ('04, '04 a) was the first to observe a true microsporidian parasite (*Thelohania legeri* Hesse) of the insects under consideration. The species infects the adipose cells of the larvae of *Anopheles maculipennis* collected from a marsh between Cavalière and Saint-Tropez in France. Hesse found two cases of the infection among forty larvae examined. As to the infection in adult mosquitoes, he states that he did not study this Microsporidian in adult *Anopheles*, but it did not seem doubtful that the parasites occur also in this stage, since the infected larvae did not appear to suffer from the parasites.

Stephens and Christopher ('08) mentioned the frequent presence of *Nosema* (?) in the oesophageal diverticula of the insect without giving further data.

Thus, on the whole, Microsporidia are rather rarely found as parasitic in mosquitoes, although other Diptera, as *Simulium* and *Corethra*, have been found more frequently attacked by them.

In this connection the writer may add a short review of works on Microsporidia by North American investigators. Gurley ('93, '94) does not seem to have observed any new forms from this continent, although he had studied a number of new forms of Myxosporidia from fresh-water fish inhabiting American waters. Linton ('01) recognized a Microsporidian which appears to be identical with *Glugea stephani* (Hagenmuller) Woodcock in the intestinal wall of *Pseudopleuronectes americanus* from Katama Bay. This same parasite was also observed later by Mavor ('15), who mentioned that 50 per cent of the above-mentioned fish in the Woods Hole region were infected by the species, and that *Osmerus mordax* from the same locality was frequently infected by apparently the same form. None of the authors extended his observations upon the biological features of the organisms. *Nosema geophili*, an extracellular intestinal parasite of *Geophilus*, sp., observed and described by Crawley from Boston, is a very doubtful form, and may possibly not belong to Microsporidia.

Strickland ('13) studied a number of species of *Glugea* from *Simulium* larvae in the vicinity of Boston, described *Glugea bracteata*, *G. fibrata*, *G. multispora*, and one ambiguous species, and showed the importance of the study of these parasites on this continent from the standpoint of economic entomology. White ('19) published an important series of experiments in connection with the practical problems concerning nosema disease of honeybees due to the infection of *Nosema apis*.

We may, therefore, conclude that little is known about the North American Microsporidia.

## METHOD OF INVESTIGATION

The study has been carried on fresh material, stained smears, and section preparations. Fixation and staining are practically the same as those (Kudo, '16) which were proved to be satisfactory for the study of *Nosema bombycis*. Schaudinn's corrosive-alcohol-acetic-acid mixture gave the best result, combined with Giemsa's stain, followed by acetone dehydration, and by mounting in cedar oil, Delafield's hematoxylin or Heidenhain's iron hematoxylin, counterstained with orange G. or eosine. For the extrusion of the polar filament, the writer has employed solely the pressure method (Kudo, '13), as perhydrol could not be obtained, which reagent, had it been used would have disclosed distinctly the mechanism of the filament extrusion because of the very large dimensions of one of the forms described here (Kudo, '18). For staining the extruded polar filament, Fontana's mixtures for staining *Spirochetes* were found to be well fitted as before. This method brings out not only the polar filament, but also the polar capsule of the spore as is stated later.

Experimental infection of the larvae of *Culex pipiens* by *Nosema baetis* failed to bring out any positive results. The experiment will be repeated as soon as the writer obtains the material.

## THELOHANIA MAGNA KUDO, PARASITIC IN CULEX PIPIENS

The larvae of *Culex pipiens* were collected on October 3 and 6, 1919, from a small pool filled with stagnant and muddy sewage water located on the side of the shallow stream in the drainage ditch near Crystal Lake at Urbana, Illinois. The larvae, as well as the pupae and adults which metamorphosed later from the larvae kept in the laboratory, presented the characters of *Culex pipiens*, as described by Howard, Dyer, and Knox ('15). On October 3, fourteen larvae and five pupae, and on the 6th, twenty-four larvae and eighteen pupae were obtained from the same pool, and were kept alive in glass jars in the laboratory. These larvae presented somewhat variable appearances, although they did not show any marked difference in their activity. Some of the larvae appeared to be more opaque whitish, with more or



less distended thorax, than the majority. On microscopical examination, the former proved to be infected by a microsporidian parasite represented by numerous very conspicuous spores. Hence, all the specimens were subjected to careful examinations, either in fresh smear or in section preparations.

The results of the examination are as follows (table, pp. 158 and 159).

As will be seen from the above, out of thirty-eight larvae examined, six were infected by the present parasite. Thirteen pupae examined were free from the infection. One of nine adult mosquitoes examined showed a few spores in fresh smear, while the rest were uninfected. Judging from the results of the examination recorded above, showing that almost all of the infected larvae manifested more or less heavy infection, the parasite appears to exercise a mortal effect upon the host, and the infected larvae die before completing their larval life. Most careful examination of the sections of the other larvae failed to reveal any with slight infection. As the habit of the larvae shows, those infected either swallowed a large amount of infected tissue of the larva dead from the infection and underwent decay at the bottom of the pool, so that the heavy infection resulted in comparatively short time, or became infected when quite young. Although the writer did not encounter any larva slightly infected, there would most probably be some larvae which, having only been infected to a slight degree or at later days of the larval stage, may be able to transform into pupae and even into adult mosquitoes. In order to solve these questions the writer went to the place on November 17, only to find that heavy and continuous rain in the latter part of October connected the pool with the main stream, producing a suitable place for small fishes, so that not a single mosquito larva was found.

The following description of schizogony and sporogony is the summary of observations on a single section preparation and a number of smears.

The youngest schizonts found in the adipose cell are rounded bodies, each containing a deeply staining nucleus (figs. 1, 2). They are about 4 to 5 $\mu$  in average diameter. The nucleus is usually located in the center. These schizonts are grouped in the

NUMBER OF LARVA	DATE OF COLLECTION	DATE OF EXAMINATION	CONDITIONS OF LARVA, WHEN EXAMINED OR FIXED	METHOD OF EXAMINATION	RESULTS OF OBSERVATION
1	Oct. 3	Oct. 4	Alive, thorax opaque	Fresh smear	Spores abundant
2	Oct. 3	Oct. 4	Dead, opaque	Fresh smear	Spores abundant
3	Oct. 3	Oct. 4	Dead, opaque	Fresh smear	No Microsporidia
4	Oct. 3	Oct. 4	Alive, opaque	Fresh smear	No Microsporidia
5	Oct. 3	Oct. 4	Alive	Fresh smear	No Microsporidia
6	Oct. 3	Oct. 4	Alive, opaque	Fresh smear	Spores fairly abundant
7	Oct. 3	Oct. 4	Dead	Fresh smear	No Microsporidia
8	Oct. 3	Oct. 6	Dead	Fresh smear	No Microsporidia
9	Oct. 3	Oct. 6	Alive	Fresh smear	No Microsporidia
10	Oct. 3	Oct. 15	Alive	Section	No Microsporidia
11	Oct. 3	Oct. 15	Alive	Section	Heavy infection
12	Oct. 3	Oct. 20	Alive	Section	No Microsporidia
13	Oct. 3	Oct. 20	Alive	Section	No Microsporidia
14	Oct. 3	Oct. 20	Alive	Section	No Microsporidia
15	Oct. 6	Oct. 7	Alive	Fresh smear	No Microsporidia
16	Oct. 6	Oct. 7	Alive	Fresh smear	No Microsporidia
17	Oct. 6	Oct. 7	Dead	Fresh smear	No Microsporidia
18	Oct. 6	Oct. 7	Dead	Fresh smear	Numerous spores
19	Oct. 6	Oct. 7	Dead	Fresh smear	No Microsporidia
20	Oct. 6	Oct. 7	Alive	Fresh smear	No Microsporidia
21	Oct. 6	Oct. 7	Dead	Fresh smear	No Microsporidia
22	Oct. 6	Oct. 7	Dead	Fresh smear	Parasitic masses visible
23	Oct. 6	Oct. 8	Dead	Fresh smear	No Microsporidia
24	Oct. 6	Oct. 8	Dead	Fresh smear	No Microsporidia
25	Oct. 6	Oct. 8	Alive	Fresh smear	No Microsporidia
26	Oct. 6	Oct. 8	Alive	Fresh smear	No Microsporidia
27	Oct. 6	Oct. 8	Dead	Fresh smear	No Microsporidia
28	Oct. 6	Oct. 8	Dead	Fresh smear	No Microsporidia
29	Oct. 6	Oct. 9	Dead	Fresh smear	No Microsporidia
30	Oct. 6	Oct. 9	Dead	Fresh smear	No Microsporidia
31	Oct. 6	Oct. 9	Dead	Fresh smear	No Microsporidia
32	Oct. 6	Oct. 9	Dead	Fresh smear	No Microsporidia
33	Oct. 6	Oct. 9	Dead	Fresh smear	No Microsporidia
34	Oct. 6	Oct. 21	Dead	Section	No Microsporidia
35	Oct. 6	Oct. 21	Dead	Section	No Microsporidia
36	Oct. 6	Oct. 21	Dead	Section	No Microsporidia
37	Oct. 6	Oct. 21	Dead	Section	No Microsporidia
38	Oct. 6	Oct. 21	Dead	Section	No Microsporidia

NUMBER OF PUPA	DATE OF COLLECTION	DATE OF EXAMINATION	CONDITIONS OF LARVA, WHEN EXAMINED OR FIXED	METHOD OF EXAMINATION	RESULTS OF OBSERVATION
1	Oct. 3	Oct. 4	Alive	Fresh smear	No Microsporidia
2	Oct. 3	Oct. 4	Alive	Fresh smear	No Microsporidia
3	Oct. 3	Oct. 4	Alive	Fresh smear	No Microsporidia
4	Oct. 3	Oct. 4	Alive	Fresh smear	No Microsporidia
5	Oct. 3	Oct. 4	Alive	Fresh smear	No Microsporidia
6	Oct. 6	Oct. 7	Alive	Fresh smear	No Microsporidia
7	Oct. 6	Oct. 7	Alive	Fresh smear	No Microsporidia
8	Oct. 6	Oct. 7	Alive	Fresh smear	No Microsporidia
9	Oct. 6	Oct. 22	Alive	Section	No Microsporidia
10	Oct. 6	Oct. 22	Alive	Section	No Microsporidia
11	Oct. 6	Oct. 23	Alive	Section	No Microsporidia
12	Oct. 6	Oct. 23	Alive	Section	No Microsporidia
13	Oct. 6	Oct. 23	Alive	Section	No Microsporidia
<b>NUMBER OF ADULT</b>					
1	Oct. 6	Oct. 13	Alive	Fresh smear	No Microsporidia
2	Oct. 6	Oct. 13	Alive	Fresh smear	A few spores
3	Oct. 6	Oct. 13	Alive	Fresh smear	No Microsporidia
4	Oct. 6	Oct. 13	Alive	Fresh smear	No Microsporidia
5	Oct. 6	Oct. 13	Alive	Fresh smear	No Microsporidia
6	Oct. 6	Oct. 24	Alive	Section	No Microsporidia
7	Oct. 6	Oct. 24	Alive	Section	No Microsporidia
8	Oct. 6	Oct. 24	Alive	Section	No Microsporidia
9	Oct. 6	Oct. 24	Alive	Section	No Microsporidia

peripheral region of the host cell where the spore formation has not yet taken place. The nucleus, in most cases, stains deeply without showing any particular details, but appears as a compact rounded mass (figs. 1, 2). The schizonts grow at the expense of the reserve material of the host cell.

The schizont multiplies by either binary fission or multiple division. The nucleus assumes an irregularly coiled thread form (fig. 3), divides into two (figs. 5, 6), and completes the division (figs. 7, 8, 9). The body elongates and divides into two, each containing a single nucleus (figs. 8, 9). Frequently the further division of the two daughter nuclei takes place without being accompanied by the separation of the protoplasm. There are thus formed tetranucleate or octonucleate schizonts (figs. 11, 14), which give rise to four or eight uninucleate daughter schiz-

onts, respectively. The schizonts very frequently become larger and elongated, assuming oblong shape with a large nucleus (fig. 15). The protoplasm in such a form is more dense, although it is equally finely reticulated as in rounded forms. The nucleus undergoes division, while the body becomes more and more elongated, often assuming a spindle shape (figs. 16 to 19). After the completion of the division, the daughter nuclei, which seem to be rich in nuclear fluid, move toward the opposite extremities of the body. The constriction in the middle of the body becomes deeper (figs. 20, 22), and finally the body separates into two. The resulting forms are more or less elongated uninucleate sporonts (fig. 23).

The schizogony observed in the present form, as is stated above, is, on the whole, similar to that of *Thelohania maenadis* which was described by Pérez ('05). Although in many other species repeated binary fission has been reported, without the complete separation of the daughter cells, producing multinucleate sausage or rosary forms, it was not observed in the present case. The multinucleate forms have been recognized in the schizogony of *Thelohania mülleri* (Stempell, '02), *Thelohania chaetogastris* (Schröder, '09), and *Thelohania varians* (Debaisieux, '13). In *Thelohania corethrae*, Schuberg and Rodriguez ('15) saw only a schizogonic multiplication into eight daughter cells.

As to the nuclear division during schizogony, amitosis seems to have generally been observed in the majority of Microsporidia, mitosis having only been reported in a few cases. Pérez ('05) observed that the chromatic substance of the nucleus of the schizont of *Thelohania maenadis* became differentiated into eight Y-shaped masses, and that after passing through a 'spireme' stage, the chromosomes were divided into two groups, which change he called a typical karyokinesis. Debaisieux ('13) apparently observed a promitotic type of nuclear division in the schizogony of *Thelohania varians*, which is highly different from any ordinary amitosis, although he described it as amitotic division. In other Microsporidia, investigators agree in reporting the nuclear change as a direct division, because of the minuteness of the object.

The nuclear division during schizogony in the present species is not of as simple a type as was observed by the writer ('16) in *Nosema bombycis* or in *Nosema baetis* described below. The chromatic substance seems to undergo a thread formation (fig. 3) which finally becomes divided into two groups (figs. 5, 6). No achromatic figures were recognizable. Further data are missing on account of rather advanced state of the parasites. Debaisieux seems to have noticed a similar phase of the nuclear change (compare his figures, especially fig. 48, with fig. 6 of the present paper).

The sporont is a spherical or slightly elongated body and its protoplasm is more or less vacuolated than that of the schizont. Its nucleus divides into two (figs. 25 to 27), the body remaining rounded. The nuclei further divide simultaneously, producing four nuclei (figs. 28, 29). The protoplasm divides also into four pyriform bodies which are connected at the center of the sporont (figs. 30, 32, 33). They sometimes cease to undergo further division and become rounded sporoblasts (fig. 31). In most cases, however, these four cells, still connected, undergo further division. Each nucleus divides once more in a plane at right angles to the longitudinal axis of the body, and the protoplasm splits longitudinally into two. Although no division of the body into eight sporoblasts was actually seen, stages such as shown in figure 33, in which one of the nuclei is dividing into two, seem to justify this statement. Thus finally eight sporoblasts become differentiated which remain united for some time (fig. 35). During these changes, the nucleus is located at the distal end in each dividing form.

Thus the sporont produces eight (sometimes four) sporoblasts, which are connected by the central mass of protoplasm for some time after the completion of division.

The sporoblast is an oblong body (fig. 36). The nucleus is usually near the rounded extremity, later moving toward the center of the body. It is generally composed of numerous granules which stain very deeply. When the sporoblast begins to transform into a spore, the nucleus divides into two of unequal size. The smaller nucleus is found near one of the extremities, and

appears as a single mass (figs. 41, 42) or more frequently an aggregation of three or four deeply staining compact granules (figs. 37 to 40). The larger nucleus, which is located near the center of the sporoblast, appears to be vesicular and shows chromatic granules chiefly attached to the network and the nuclear membrane (figs. 37 to 40). The sporoblast, in the meantime, assumes an elongated shape. In the attenuated part, the protoplasm becomes highly vacuolated, and develops into the polar filament (figs. 40 to 49). In the smear preparation, the sporoblast at this stage exhibits a clear but narrow longitudinal but not straight space (figs. 41 to 43), which becomes broader as the formation of the polar filament progresses, since the polar filament becomes coiled close to the polar capsule which develops at the same time. In sections of young spores, on the other hand, one sees either transverse lines of the coiled filament across the capsule, when they are sectioned near the surface (figs. 47, 50, 51) or dots of uniform size scattered in the capsule when the spore is sectioned through its center (figs. 48, 49). The spore membrane becomes differentiated around the spore, for which no nucleus could be recognized. The sporoplasm occupies rounded portion of the spore.

As far as the writer is aware, the copulation of isogametes or autogamous union of the two nuclei at the end of schizogony which produces copula or zygote, a sporont, has been reported but by two authors. Mercier ('08 a, '09) observed a caryogamy in the development of *Thelohania giardi*, which produced a copula, the sporont. Debaisieux ('13) on the other hand, recognized an autogamy at the end of the schizogony of *Thelohania varians*, which formed a uninucleate zygote or the sporont. The same author ('15) further reported a similar process in *Glugea mülleri* and *Glugea danilewskyi*. Pérez ('05) mentioned the presence of uninucleate or binucleate Gregarine-like bodies which underwent active movements in *Thelohania maenadis*, and suggested that these forms might be the phases which belong to the process of fecundation.

In the present form, the writer has observed forms (figs. 15, 18) which apparently correspond with those observed by Pérez

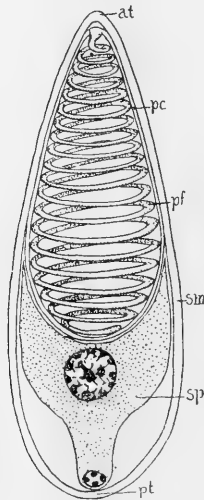
in *Thelohania maenadis* and by Debaisieux in *T. varians*. Yet stages like figures 16, 17, 20, and 21 lead the writer to consider them as stages of division instead of fusion of two forms. The writer once noticed such a form as is shown in figure 24, which may be considered as a preliminary stage of copulation of two isogametes. However, he has no further evidence of the fusion of gametes or the union of two nuclei in the present form.

The writer had already stated that there appears a narrow space in the developing sporoblast, and that it becomes broader as the formation of the polar filament progresses. Debaisieux ('15) saw probably a similar structure ('canalicule') in the spore of *Glugea mülleri*, but interpreted the meaning in an entirely different manner.

The fully mature spore is 12 to  $13.5\mu$  long and  $4\mu$  broad. It is elongated pyriform and usually bent toward one side. In cross-section it is circular. The posterior end is broadly rounded, while the anterior extremity is less rounded, though not attenuated.

In fresh state, the mature spore shows a great vacuolation in its anterior portion (fig. 56). For about two-thirds its length from the anterior tip, fine irregular lines of coiled filament are seen, which become more distinctly visible when the spore is stained; while the posterior third is occupied by a finely granulated protoplasmic mass which often contains a refringent body near its extremity. When the fresh spores are subjected to mechanical pressure and are stained by Fontana's mixtures, the extruded polar filament is distinctly recognizable (fig. 57). It is uniformly fine and reaches a length of  $230\mu$ . The writer does not think this the average length, but records it here as the longest one found so far. The average length varies from 150 to  $200\mu$ . This particular spore (fig. 57) shows not only the extruded filament, but also distinctly its remaining part spirally coiled inside of the capsule. The same figure at the same time gives a strong basis on the presence of a polar capsule containing a spirally coiled polar filament. The spore membrane does not exhibit any sutural line as was recognized in the spore of *Thelohania giardi* (Thélohan, '95) or of *Thelohania* sp. (Mercier, '08).

When fixed and stained, the spore shows its structure more distinctly. Inside of the spore membrane, a large pyriform polar capsule becomes visible together with the filament coiled within it. The polar capsule, 6 to  $7.5\mu$  long, occupies half or two-thirds of the anterior portion of the spore (figs. 50, 51, 54). The foramen of the capsule can not be seen well, but the fact that the polar capsule opens at the anterior tip of the spore is clearly perceived in the spore in figure 57. The wall of the polar capsule



Text figure A. A schematic representation of a spore of *Thelohania magna* *at*, anterior tip; *pc*, polar capsule; *pf*, polar filament; *pt*, posterior end; *sm*, spore membrane; *sp*, sporeplasm. About  $\times 5000$ .

is comparatively thin and is very faintly stained in many cases with Giemsa's mixture. In deeply colored spores, however, it is distinctly recognizable as a reddish sac. In younger spores it is evident, but it is more distinctly visible when the spore is brought under mechanical pressure.

The polar filament is coiled spirally along the inner surface of the polar capsule (figs. 53 to 55, 57). The spiral begins at the anterior tip of the capsule, and does not differentiate any central axis. Figures 43 and 47 show the developing polar capsule with its filament in young spores, the windings are more or less clearly



visible. Three different views of a stained spore in a smear preparation are shown in figures 53 to 55, which exhibit the spiral course plainly.

The sporoplasm occupies the posterior portion of the spore. In fixed preparations, a clear space appears around its side (figs. 48 to 55). Two nuclei occur, one large vesicular body, usually with one or two karyosomes, is located close to the capsule, and the other is near the posterior extremity of the spore, as was stated before.

As to the structure of the spore of *Microsporidia*, observations of various investigators differ to a greater or less extent. These will be briefly reviewed below, although the writer ('16, '20) has already discussed them in his previous papers.

A more or less generally accepted conception of the structure of the microsporidian spores was proposed by Mercier ('08 a, '09) for *Thelohania giardi*. He observed that the spore is covered with a bivalve shell, each valve developing from a uninucleate parietal cell; that the spirally coiled filament is contained in a polar capsule which has its nucleus; that the girdle-shaped sporoplasm with at first two, later four nuclei, surrounds the polar capsule, and that a vacuole is present at each pole of the spore. With this view, on the whole, Schröder ('09), Stempell ('09), Fantham and Porter ('12, '14), Strickland ('13), Kudo ('16), and others in other *Microsporidia*, agree, although their observations differ in details.

On the other hand, Schuberg ('10) noticed in the spore of *Plistophora longifilis* that the girdle-shaped sporoplasm (which is ring-like in cross-section) contains only a single nucleus; that the filament is coiled directly under the shell, mostly in the posterior portion of the intrasporal space; that the nuclei observed by other investigators are none other than the metachromatic granules. The same view has been maintained by Weissenberg ('11, '13), Omori ('12), and Debaisieux ('13, '15).

Léger and Hesse ('16) described an interesting group of *Microsporidia* under the generic name of *Mrazekia*. The spores are of cylindrical form and have an entirely different structure from other known genera. The polar filament is differentiated into

two parts. No polar capsule is mentioned, the filament being coiled directly inside of the shell. The binucleate sporoplasm, instead of being girdle-shaped is a rounded and more or less well-defined body imbedded in a clear space at the posterior part of the spore.

The same authors ('16 a) later reported a similar observation on the structure of the spore of *Plistophora macrospora*. They stated that the polar capsule lies close to the shell, occupying the greater part of the intrasporal space; that the filament is coiled in the capsule without a central axis; that the sporoplasm is a rounded binucleate body imbedded in the posterior vacuole of the spore; that the girdle-shaped structure, thought by numerous authors to be the sporoplasm, is none other than the retracted substance composing the polar capsule, so that one or two turns of the filament were mistaken in optical sections as a variable number of nuclei, and that the granule in the posterior vacuole, which was regarded as a metachromatic granule by some authors, is really the nucleus of the true sporoplasm. Georgévitch ('17) agreed with this view in his study on *Nosema (Glugea) marionis*, although he noticed that some spores did not contain any polar capsule.

The writer ('20) remarked that the microsporidian spores do not seem to be unique in structure, and that they may be classified into at least two types: one, represented by *Thelohania magna*, the other, by *Nosema bombycis*. Studying carefully the forms which are described in the paper, he strongly maintains this view. To this first type may belong *Thelohania varians* (Debaisieux, '13), *Plistophora elegans* (Auerbach, '10 a), *Plistophora macrospora* (Léger and Hesse, '16 a), *Thelohania illinoensis* (see below), and *T. acuta* (Schröder, '14). The spore of this last form, according to Schröder, has a structure similar to that in *Thelohania magna*, although Schröder could not make the filament visible or extruded.

THELOHANIA ILLINOISENSIS NOV. SPEC., PARASITIC IN  
ANOPHELES PUNCTIPENNIS

Thirty-four larvae of *Anopheles punctipennis* were collected from the drainage ditch at Urbana, Illinois, on September 25 and October 3, 1919. They were kept in two aquaria in the laboratory. Six from the first collection were allowed to complete their metamorphosis. Among the remaining twenty-eight, one individual was more opaque in color and less active in motion than others, and proved, on microscopical examination, to be infected by a Microsporidian, to which the name *Thelohania illinoisensis* is given. The remaining larvae were subjected to careful examination. Only one of these was found infected. Unfortunately, the number of parasitized larvae was small and none of the sectioned larvae was infected. The two infected larvae were studied in fresh as well as in fixed and stained smears.

The adipose tissue seems to be the only seat of infection. Whether or not other tissues are affected could not be determined, because the sectioned larvae contained no parasites. The smears showed mainly isolated spores and sporonts with young spores. The generic position of the parasite in the genus *Thelohania* is, however, without doubt, because of the abundant presence of octosporous sporonts. The schizogony as well as sporogony could not be studied, as young stages were very few in number.

The sporont with eight spores was usually rounded (fig. 61) or rarely oblong (fig. 69). The spores were dispersed without any order inside of the sporont membrane and were imbedded in faintly staining protoplasm. The rounded sporonts with mature eight spores measure  $13\mu$  in average diameter, while oblong forms measure  $16\mu$  by  $8\mu$ .

The spore is oval in form with equally rounded extremities. It is less refractive than that of *Nosema bombycis* or *Nosema apis*. It is uniform in size, no dimorphism of spores being found.

In fresh state, the spore membrane is clearly seen separated from its contents (figs. 62, 63). The contents of the spore are very peculiar in shape; one end narrow and truncate, the other regularly rounded along the inner surface of the membrane.

The contents are either uniformly granular (fig. 62) or with a vacuole, rounded triangular in form (fig. 63). Thus the appearance of fresh spores differs from that of *Thelohania magna*, *Nosema bombycis*, *N. apis*, or *N. baetis*. The mature spores are 4.75 to 6 $\mu$  long and 3 to 4 $\mu$  broad.

In fixed preparations, the spore shows quite characteristic appearances. The spore membrane becomes truncate at the end in which the contents are (figs. 65, 67, 68). When stained with Giemsa, the comparatively thick spore membrane remains unstained, while the contents become differentiated, the cap-shaped part being stained pink red, the other part of the contents assumes a blue color, in which numerous irregular striations are seen (figs. 64 to 66, 69). Near the latter mass there always appears in the pinkish protoplasm one or two spherical granules which stain a deep red (fig. 69). The pink mass with the deeply staining granules is most probably the sporoplasm, and the bluish colored mass apparently the polar capsule with a coiled polar filament. When the spores are stained with Heidenhain's iron hematoxylin, they assume different aspects according to the degrees of decoloration. In more or less deeply stained spores, the oval polar capsule is brought into view (fig. 67). In well-differentiated spores, however, one can recognize similar structure found in Giemsa stained spore (fig. 68).

In *Thelohania legeri* Hesse, to which the present form is undoubtedly closely related, a somewhat similar structure was noticed by Hesse ('04). He describes observations upon stained spores as follows:

L'hématoxyline ferrique colore dans les spores une grosse masse centrale qui représente vraisemblablement l'appareil capsulaire et le germe. La méthode de Romanovsky différencie dans cette masse centrale un amas chromatique ordinairement formé de quatre grains juxtaposés, représentant l'élément nucléaire primitif qui doit donner naissance ensuite au noyau de la capsule et à ceux du germe, ainsi qu'en témoigne sa division ultérieure, mais c'est un point que je n'ai pas encore suffisamment approfondi.

Hesse did not state from which end of the spore the filament was extruded, although he says that the anterior end was truncate. It may be understood that the anterior or truncate end is the

extremity through which the filament is extruded. This is not the case with the present form.

When the spore of the form under discussion is subjected to mechanical pressure, it extrudes its polar filament from the rounded, not from the truncate extremity. This has been distinctly demonstrated by staining the pressed spores by Fontana's mixtures (figs. 73, 74). One or two granules usually unequal in size are generally found in the empty spore membrane. These granules are most probably the nuclei of the sporoplasm which have already been mentioned (compare fig. 69 with figs. 70 to 72).

All of these observations lead the writer to consider the structure of the spore of *Thelohania illinoisensis* as follows: Inside of the comparatively thick spore membrane, a large polar capsule occupies about two-thirds of the intracapsular cavity, the remaining space is partly filled with the sporoplasm which contains one or two nuclei of unequal size. Thus on the whole, the structure of the spore of the present form is similar to that of the spore of *Thelohania magna*.

The length of the filament extruded by means of mechanical pressure and stained with Fontana's mixtures varies from 60 to 97  $\mu$ . The filament is more or less thicker than that of *Nosema bombycis* (Kudo, '13, '16), or of *N. baetis* (figs. 113, 114). The difference in length of the polar filament may be attributed partly to the unequal effect of the pressure upon the spores, but mainly is due to the fact that the filament is of various length in different spores as can be seen in each spore before the filament extrusion. The writer stated already that in fresh spores the contents appear either to be uniformly granulated or to contain a clear space near the center (figs. 62, 63). This diversity in the appearance of the contents depends upon the length of the filament developed inside. When the filament is well developed and fills the cavity of polar capsule, the spore does not show any clear space (fig. 62). On the other hand, if the polar filament is not well developed or short, it does not occupy the entire capsular cavity, but leaves a clear triangular space near the posterior end of the capsule. Usually the filament seems to be coiled in from

fifteen to twenty turns in the capsule. Figure 71 shows the extruded filaments which suggests that it was coiled seventeen times, and at the same time demonstrates the fact that the polar filament is coiled in the way as was suggested by the writer ('16) in *Nosema bombycis* and also in *Thelohania magna* (text figure), but not in the way figured by Stempell ('09), who thought the filament was coiled around a central axial portion, which conception was supported by Zander ('11) and Strickland ('13). One will notice the wavy course throughout the entire filament, the height of the wave being smallest at the extremities. This condition of the extruded filament agrees well with the oval shape of the polar capsule and the suggested arrangement of the filament in the capsule.

Of all the known species of the genus *Thelohania*, twenty-three in number, including *T. magna*, *T. legeri* Hesse is related most closely to the present form. As was stated above, this species is parasitic in the adipose cells of *Anopheles maculipennis*, and has spores of similar structure. One may be inclined to think that this form and the species recorded here are one and the same, although the former was observed in France. Indeed, except for the irregularity and slight difference in the dimensions of the spores and the difference of the host species, the two species would be distinguished from each other only with difficulty.

The difference in the length of extruded filament has not the importance in the identification of species of Microsporidia which some authors have given it (for example, Strickland, '13) because there is very often a conspicuous variation even in one and the same species. Besides, the different methods employed for the study of the filament frequently bring out entirely different results. This is best demonstrated by the filament of the spore of *Nosema bombycis* which have been studied by three investigators, each using a different method. Thélohan ('94), for the first time, proved the presence of the filament in a microsporidian spore by treating spores of *Nosema bombycis* with nitric acid, and recorded it as 12 to 14 $\mu$  long. Stempell ('09), on the other hand, used iodine alcohol, and found that the length of the filament was 32 to 34 $\mu$ . Kudo ('13, '16, '18), by using

mechanical pressure or perhydrol and staining with Löffler's or Fontana's mixtures, proved that the fully extruded filament was 57 to 72 $\mu$  long, sometimes reaching 98 $\mu$ , and at the same time proved that Stempell's calculation of the number of turns of the coiled filament was not correct.

Hesse caused the extrusion of the filament in *Thelohania legeri* by means of iodine water, and found it to be about 50 $\mu$  long, while the present writer used the pressure and staining method for the present parasite. This has been recognized as a reliable way of studying the filament, especially in Microsporidia and is accepted by some workers, as Erdmann ('17). Consequently, the difference in length of the filaments in the two species under discussion cannot be used as a basis of distinction.

Yet the rather conspicuous difference in size of the spores prevents the writer from assuming these two forms as identical, especially as Hesse mentioned an irregularity of the dimensions, which is not the case in the American form. Hesse states that the spores of *Thelohania legeri* measure generally 8 $\mu$  by 4 $\mu$ , that in certain sporonts they measure only 6 $\mu$  by 3 $\mu$ , and in some macrospores 12 $\mu$  by 5 $\mu$ . The spores of American species are uniformly much smaller, being 4.75 to 6 $\mu$  by 3 to 4 $\mu$ , without any dimorphism or regular abnormality of the spore.

Hence, the writer thinks that the species under consideration is not identical with the closely allied *Thelohania legeri* Hesse, and is a new form which has not been recorded before. He, therefore, names it *Thelohania illinoisensis*.

NOSEMA BAETIS NOV. SPEC., PARASITIC IN BAETIS SP.(?)<sup>2</sup>

Forty-two nymphs of *Baetis* sp. (?) were collected in the drainage ditch at Urbana, Illinois, on September 25, October 3, and November 17, 1919, and were kept in an aquarium in the laboratory. Six out of twenty-four nymphs collected on the first two dates and two out of eighteen of the last collection were infected by a Microsporidian, for which the name *Nosema baetis* nov. spec. is proposed.

<sup>2</sup> The writer is indebted to Mr. J. R. Malloch, of the Illinois Natural History Survey, for the generic identification of the insect.

The parasites attack the adipose cells, all other tissues remaining uninfected. The thorax of the infected nymph was strikingly whitish opaque, and was more or less distended, compared with that of normal individuals. Although the normal insects swam away very rapidly from anything put into the water, the infected ones showing the above-mentioned external characters, were caught easily by means of forceps. The decrease in activity of the host insect is undoubtedly due to the decrease of muscular activity, which relation is discussed elsewhere (p. 176).

As the number of these insects was small, while on the other hand, a large number of newly hatched larvae of *Culex pipiens* was available, the latter were used for artificial infection. A number of three-day-old larvae of *Culex pipiens* were reared in an aquarium which was filled with water suspension of the microsporidian, made from an infected *Baetis* nymph. The larvae seemed to eat willingly the small fragments of the infected tissue. Four larvae were fixed daily until the tenth day, none dying during this period. The examination of sections of these experimental larvae failed to reveal any infection whatsoever. Abundant spores which were found in the alimentary tract of the larvae did not show any recognizable changes in their internal structure. From this experiment it may be stated that *Nosema baetis* does not infect three-day-old larvae of *Culex pipiens* in the laboratory.

The following observations on its schizogony and sporogony have been carried out in smears and section preparations of naturally infected *Baetis* nymph.

The youngest intracellular stage, the schizont, was found in the adipose cell. It is a small rounded body about  $3\mu$  in diameter, and contains a comparatively large nucleus, surrounded by a clear and narrow space (fig. 75). In general appearance it resembles *Nosema bombycis* (Kudo, '16). The protoplasm is densely granulated, staining bright blue with Giemsa. The schizont multiplies by binary fission. The nuclear division seems to be amitotic; the nucleus simply divides into two parts, at first separated by a narrow space (figs. 76, 77); later these two portions move toward the opposite poles, often showing an arch shape (figs. 76 to 93). The daughter schizonts repeat the division until the host cell becomes filled with the sporonts.



Multiple or delayed binary fission was not observed, which besides binary fission is a very common process in the genus *Nosema*.

Young rounded sporonts become elongated and the protoplasm condenses either toward one end or to the center of the sporont (figs. 94 to 99). In most cases it assumes a girdle shape at the middle of the spore, which shows a ring form in cross-section.

The polar capsule seems to become differentiated nearly in the center of the spore, best seen in spores stained with Fontana's mixtures (figs. 109 to 112). A nucleus for the spore membrane was not observed. Young spores (figs. 104, 105) are slightly larger than the mature ones (figs. 107, 108). These two stages show different affinities toward stains. Heidenhain's iron hematoxylin stains young spores usually dark black, leaving a small clear space near the narrow tip, where a median longitudinal line was seen (fig. 104), while the smaller and mature spores are stained very faintly, even without differentiating the nucleus (fig. 107). Giemsa stains the former uniformly pinkish, with a centrally located deep red mass which appears in various forms (fig. 105), but the latter is stained very faintly, exhibiting only a blue mass near the center (fig. 108).

One might think that these two forms correspond to the macrospore and microspore, respectively, which have been reported to occur in several other forms. It is, however, the writer's opinion that they only differ in development, as various intermediate forms between them show the gradation from the larger and younger spores to the smaller and mature ones.

The mature spore is 3 to 4 $\mu$  in length and 1.5 to 2.5 $\mu$  in breadth. The polar filament, extruded and studied by exactly the same method used for the two other forms, was 94 to 135 $\mu$  in length (figs. 113, 114).

The structure of the spore seems to be similar to that of *Nosema bombycis* (Stempell, '09, and Kudo, '16), which was proposed as the representative of the second type of microsporidian spores (Kudo, '20). When mature the spore becomes covered with a comparatively thick membrane which prevents the stains from acting upon its contents. The polar capsule occupies the

central portion of the spore cavity, being connected to the spore membrane at one of the extremities (figs. 109, 110). The sporoplasm surrounds the polar capsule at the middle part of the spore (figs. 105, 108). Unfortunately, the nucleus did not stain satisfactorily.

Thus we have here another example of a spore possessing a structure similar to that of *Nosema bombycis*. To this group may possibly belong *Nosema apis* (Zander, '11, and Fantham and Porter, '12), *N. bombi* (Fantham and Porter, '14), and *N. sp.* (Ishiwata, '17).

As quoted before, Léger and Hesse ('16 a) suggested that all the microsporidian spores are of similar structure to those represented by those of *Plistophora macrospora* and of the genus *Mrazekia*. In this connection, regarding the present writer's observation ('16) on *Nosema bombycis*, they wrote as follows: "La taille (of the free amoeboid mass) en est très exigue ( $1\mu$  à  $1.5\mu^3$ ), alors que, dans les spores mûres, qu'il dessine à la même échelle, il donne comme germe l'anneau colorable capsulaire dont les dimensions sont de beaucoup supérieures (comparer ses fig. 35 et 37, Pl. I.)." It should, however, be easily understood that this difference in size does not give any strong basis for interpreting their generalization of the structure of various spores of Microsporidia, if one consider the fact that there is an irregularity in size among spores of *Nosema bombycis* as well as those of other forms to a certain extent; and also that two phases of the sporoplasm differ in shape, i.e., the sporoplasm assumes a ring form around the polar capsule, whereas the free amoeboid mass outside of the spore is a solid mass.

Up to the present, seven species of Microsporidia have been reported to be parasitic in Ephemeridae. Hesse ('03) observed *Gurleya legeri* parasitic in the adipose cell, musculature, and connective tissue of the larva of *Ephemerella ignata*. The same author ('05) reported later *Nosema vayssierei*, parasitic in the adipose cell of the larva of *Baetis rhodani*. Lutz and Splendore ('08) described briefly *Nosema ephemeræ*  $\alpha$  and *Nosema ephemeræ*  $\beta$  from the intestine of the larva of an Ephemerid insect.

<sup>3</sup> Misprinted as ' $15\mu$ .'

Finally, Léger and Hesse ('10) recorded three Microsporidia parasitic in the larvae of *Ephemera vulgata*, i.e., *Nosema schneideri*, from the epithelium of the intestine, and *Stempellia mutabilis* and *Telomyxa glugeiformis* in the adipose cells.

Of all these, *Nosema vayssierei*, *N. schneideri*, and *N. ephemeræ*  $\alpha$  stand in close relation with the present form in the general form and dimensions of the spores.

Lutz and Splendore described *Nosema ephemeræ*  $\alpha$  as follows: "Im Darne von Ephemeridenlarven fanden sich in diffusen Verbreitung eiförmige glänzende Sporen, die am stumpferen Hinterende nur selten eine Vakuole zeigen. Länge 3.5 to 4 $\mu$ , Breite 2 to 2.5 $\mu$ ." The description is too brief, but this species, even if it belongs to *Nosema*, probably differs from the present form, because of the difference of location.

*Nosema schneideri* is described by Léger and Hesse as follows:

Le *Nosema schneideri* se développe dans cellules épithéliales de l'intestin moyen de la larve d'Ephémère qu'il envahit parfois en totalité et où il évolue selon le type monosporé qui caractérise ce genre. Les schizontes sphériques, de 2 $\mu$  de diamètre, se multiplient activement par division binaire et finalement la cellule est remplie de sporontes monosporés et de spores qui la distendent. Puis les spores mûres tombent par paquets dans la cavité intestinale. Ces spores sont ovoïdes, de 4 $\mu$  sur 2 $\mu$ , avec un long filament de 90 $\mu$ . Le pôle par lequel s'échappe le filament montre une petite calotte chromatique. Le parasite ne semble pas provoquer une hypertrophie notable de la cellule hôte dont il respecte le noyau.

No figure accompanies the description. The species attacks only the epithelial cells of the intestine, and not the adipose cells, which are the only seat of infection in the present Microsporidian. It, therefore, seems to be correct to separate these two forms which resemble each other by the schizogony, sporogony, and dimensions of the spore.

Finally, *Nosema vayssierei* is described by Hesse as follows:

Ces spores sont contenues en nombre variable, souvent assez élevé, dans des sporoblastes ovalaires ayant de 6 à 9 $\mu$  de large sur 9 à 12 $\mu$  long, ou sphérique de 8 à 10 $\mu$  de diamètre. Elles sont piriformes et présentent 3 à 4 $\mu$  de grand axe sur 1 à 2 $\mu$  dans leur plus grande largeur . . . . La longueur du filament deroule atteint de 17 à 19 $\mu$ .

As Hesse saw the presence of polysporous sporonts, the species may possibly belong to another genus and not to *Nosema*. Auerbach ('10) placed this form into the genus *Glugea*.

The writer, therefore, thinks that the Microsporidian under consideration has not been observed before, and names it *Nosema baetis*.

#### THE EFFECT OF THE PARASITES UPON THE HOSTS

As far as the observations up to the present are concerned, the adipose tissue is the only seat of infection in any of these three forms.

In the larvae of *Culex pipiens*, the fat bodies composing the general lining of the body wall and those lying freely in the body cavity are heavily infected. The intermuscular adipose tissue remains uninfected. This naturally explains why the infected larvae were as active as the normal ones.

Two cases of the presence of *Thelohania illinoisensis* were studied only in smears; they do not furnish any data to be discussed here.

In the nymphs of *Baetis* sp., infected with *Nosema baetis*, the adipose tissue did not escape infection in any part of the body. The muscular tissue in most cases was pushed aside by the immense growth of the entire infected fat bodies, and showed atrophy or poor development even though the effect was indirect. In sections of a normal nymph, there are not only greatly vacuolated adipose cells, but also wide clear spaces in the body cavity between tissues. In sections of heavily infected nymphs, however, the space of the body cavity was almost entirely filled with greatly distended adipose cells which, when the animal was alive, appeared as opaque white masses. The degeneration and the dislocation of the muscular tissue and consequently less muscular activity would plainly explain why the infected nymphs were less active and more easily caught in the aquaria than the healthy ones.

Aside from such a form as *Nosema bombycis* which attacks every non-chitinous tissue of *Bombyx mori*, the great majority of Microsporidia are confined to a particular tissue of the host.

Among insects, the adipose tissue is known as the most common seat of infection. This peculiar nature of Microsporidia does not seem to have been explained. In almost all cases Microsporidia were successfully transmitted from one host to another by artificial infection per os. So it is strange to find uninfected epithelial cells of the digestive tract, through or between which the amoeboid sporoplasm must have passed into the body cavity to reach the adipose tissue after having left the spore membrane in the lumen of the alimentary canal.

The nucleus of the infected host cell of *Culex pipiens* becomes hypertrophied. The nucleus of the normal fat body is usually a small, irregularly outlined, rounded body with a largest diameter less than  $8\mu$ , and is compactly filled with deeply staining chromatic granules (fig. 59). The nucleus of heavily infected host cell, however, becomes considerably hypertrophied, the diameter reaching from 25 to  $30\mu$  (fig. 60). It also becomes vesicular and contains deeply staining irregular chromatic masses which are connected with one another and with the nuclear membrane. It further shows one or two large masses.

The infected adipose tissue of the *Baetis* nymph contained numerous hypertrophied nuclei (figs. 116, 117), much larger in number than the normal ones (fig. 115). Apparently dividing nuclei have more frequently been observed in infected than in normal tissue (fig. 117). The nucleus of the normal cell varies from 4 to  $10\mu$  in diameter, while those of the infected cells reach  $25\mu$  in diameter. The large chromatic masses in the latter stain less deeply by Giemsa than those small ones in the former.

Although the nucleus of the host cell is not actually infected by the parasites, its hypertrophy has been noticed in several cases of the microsporidian infection. One of the most striking cases was reported by Schuberg ('10) in the testicular cell of *Barbus fluviatilis* infected by *Plistophora longifilis*.

Abnormal mitotic divisions of the nucleus of fat body due to microsporidian infection were reported by Mercier ('08) and Debaisieux ('13). The active amitotic division of the nucleus, distinctly noticeable in the present case, is most probably caused by the parasites, and further, may be considered as a reaction of the host cell against the parasite.

Further reaction on the part of the host tissue against the parasites was noticed in the case of the nymph of *Baetis*. The blood-cells of the infected nymph contained from one or two to several spores of *Nosema baetis*. In Giemsa stained sections, none of them harbored young stages of parasites; all of the parasites found in the blood-cell were mature spores which were plainly demonstrated by the typical staining. If the parasites attack the blood-cells, one would expect to see schizonts and young spores in some of the newly infected corpuscles. Judging from this fact, the presence of the parasites in the blood-cell must be attributed to the active phagocytosis of the host cell. Although the actual processes were not seen, it is most probable that the blood-cells take in the spores which have been liberated in the body cavity by the rupture of the cell membrane of the infected fat bodies. Similar phagocytosis was reported in four cases. Sasaki ('97) held that the presence of spores of *Nosema bombycis* in the blood-cell of *Bombyx mori* was due to the phagocytosis of the latter. Caullery and Mesnil ('99) in *Glugea laverani*, Mrázek ('99) in *Glugea lophii*, and Weissenberg ('13) in *Glugea anomala* noticed phenomena of apparently similar nature.

#### THE SIGNIFICANCE OF THE MICROSPORIDIAN PARASITES AMONG SOME AQUATIC INSECTS

Strickland ('13) had called attention of economic entomologists to the subject in connection with the microsporidian parasites of *Simulium* larvae. Although the data which would enable the writer to discuss the subject more fully are at present insufficient, the observations which have been recorded in the previous pages lead one to consider the significance of the microsporidian infection among the aquatic larvae of some harmful insects such as mosquitoes.

If one can increase the number of cases of *Thelohania*-infection in mosquito larvae in the laboratory (which does not seem to be impossible) one may be able to use the microsporidian parasites as one of the natural enemies of mosquito larvae, by distributing the infected larval tissue in the mosquito-breeding places.

In a large body of water one can rely on fish to a greater extent for the destruction of the mosquito larvae. In a smaller body

of water where fish cannot be introduced, one may use oils for the destruction of those larvae.

Increasing the chances of *Thelohania*-infection among mosquito larvae and distributing of the infected larval tissue into the breeding place may be another method of destroying mosquito larvae in a small body of water. In case the infection be slight, the larvae, although infected, may become adults, and thus distribution of the parasites will be done by the host mosquitoes which will die on oviposition, leaving a source of infection in places which may escape our watchful eyes. The practical application, however, depends upon further study in future.

#### SUMMARY

1. Three new Microsporidia obtained in the vicinity of Urbana, Illinois, U. S. A., are described here.

2. *Thelohania magna* infects the adipose tissue of the larva of *Culex pipiens*. It is rare, and is found in a limited locality. The effect of heavy infection upon the host appears to be fatal.

3. *Thelohania illinoisensis* infects the adipose tissue of the larva of *Anopheles punctipennis*. It is rarer.

4. *Nosema baetis* attacks the adipose cells of the nymph of *Baetis* sp. It is more common than the other two.

5. Caryogamy or autogamy was not found in the sporogony in any of the three forms.

6. Two types of microsporidian spores are distinguished. One, represented by *Thelohania magna*, has a spore in which the sporoplasm occupies its posterior portion and the polar capsule the anterior part. The other, represented by *Nosema bombycis*, has a spore in which the sporoplasm surrounds the polar capsule at its middle part.

7. The hypertrophy of the nucleus of the host cell is observed.

8. The phagocytosis by the blood-cell of *Baetis* nymph of the parasites possibly occurs.

9. The fact that the polar filament cannot be used for the identification of species of Microsporidia is made clear.

10. The possibility of using microsporidian parasites as one of the means of destroying mosquito larvae is suggested.

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#### EXPLANATION OF FIGURES IN PLATES

All the drawings have been made by means of Abbe's drawing apparatus. Zeiss' compensation oculars, 6, 8, 12, and 18 and homogenous oil-immersion objective 2 mm., were used. Abbreviations in the explanations of plates are as follows:

*G.*, Giemsa-staining, followed by acetone dehydration, by mounting in cedar oil; *H.*, Heidenhain's iron-hematoxylin staining; *P.F.*, pressed mechanically and stained after Fontana's method; *S.*, section preparation; *Sm.*, smear preparation.

## PLATE 1

### EXPLANATION OF FIGURES

#### *Thelohania magna* Kudo

- 1 to 23 Stages in schizogony.
- 1 and 2 Young schizonts. S.G.  $\times$  2360.
- 3 to 6 Nuclear division. S.G.  $\times$  3500.
- 7 to 9 Successive stages of binary fission. S.G.  $\times$  2360.
- 10 to 14 Stages in multiple division. S.G.  $\times$  2360.
- 15 to 23 Stages of binary fission of the second type. S.G.  $\times$  2360.
- 24 A stage of isogamy (?). S.G.  $\times$  2360.

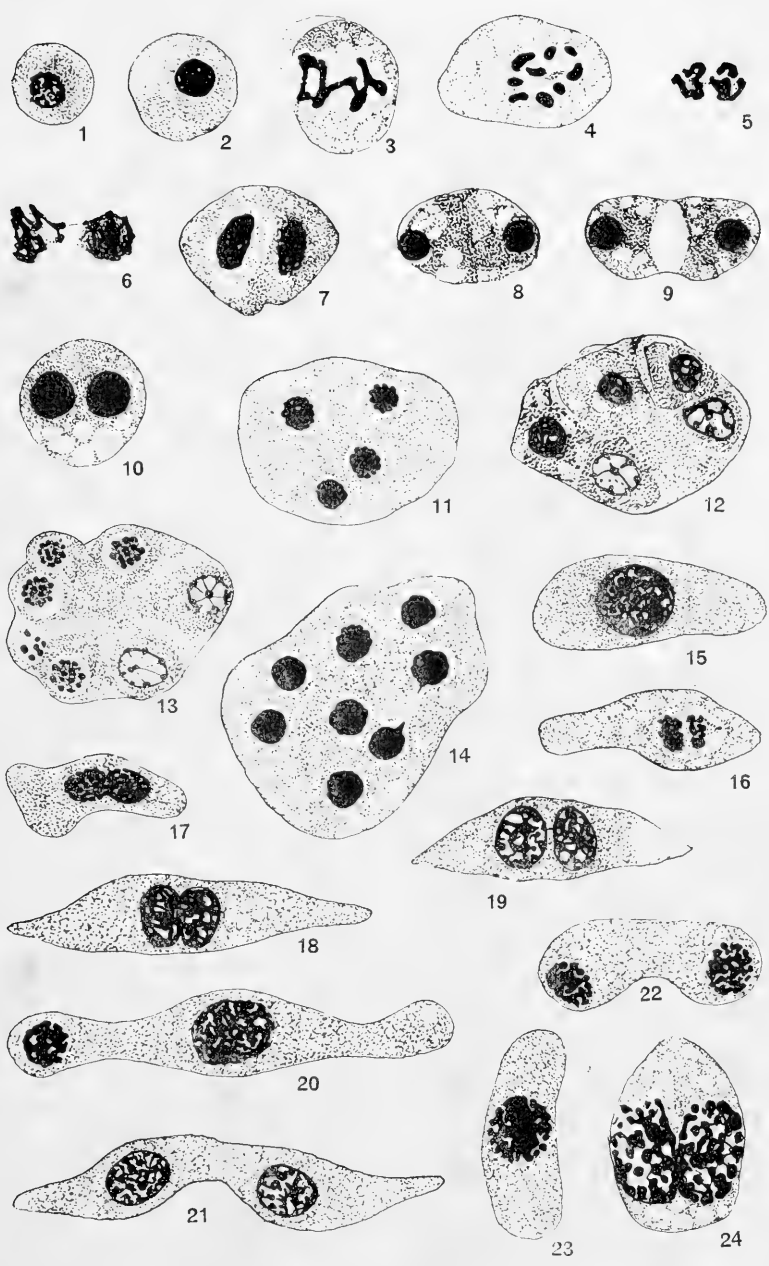
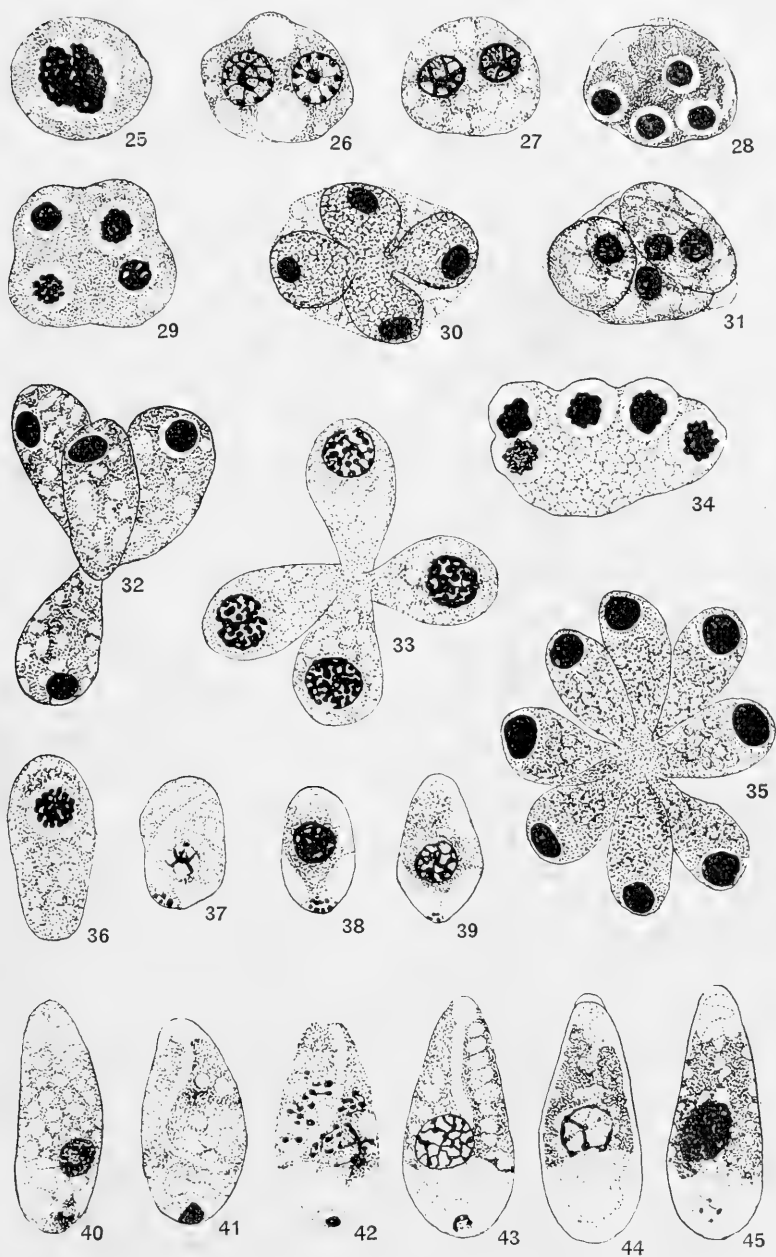


PLATE 2

EXPLANATION OF FIGURES

*Thelohania magna* Kudo

- 25 to 35 Formation of sporoblasts. S.G.  $\times 2360$   
36 to 45 Development of spore.  
36 A sporoblast. S.G.  $\times 2360$ .  
37 to 39 Further advanced stages in spore formation. S.G.  $\times 2360$ .  
40 to 45 Young spores. Sm.G.  $\times 2360$ .



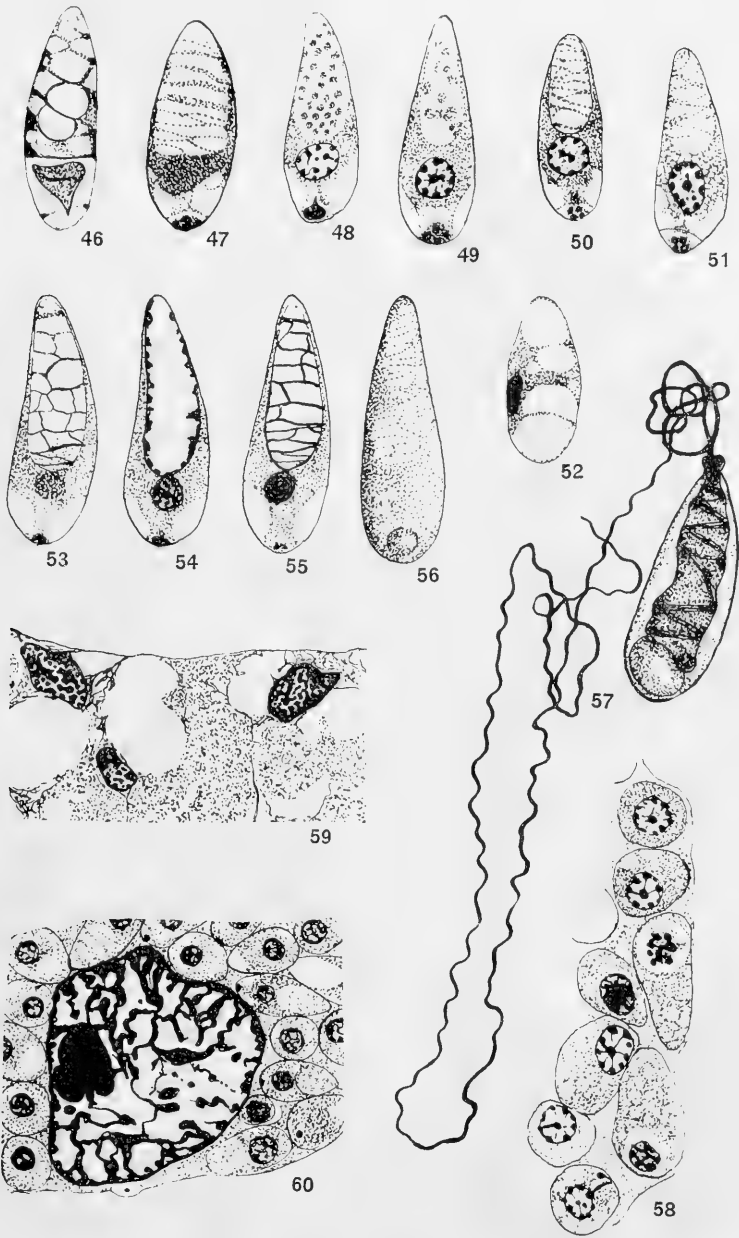
### PLATE 3

#### EXPLANATION OF FIGURES

##### *Thelohania magna* Kudo

- 46 A young spore. Sm.G.  $\times 2360$ .
- 47 to 49 Young spores. S.G.  $\times 2360$ .
- 50 and 51 Two spores sectioned near the surface. S.G.  $\times 2360$ .
- 52 An abnormal spore. S.G.  $\times 2360$ .
- 53 to 55 Three different views of a spore: fig. 53, the upper surface view; fig. 54, optical section; fig. 55, the lower surface view. Sm.G.  $\times 2360$ .
- 56 A fresh spore. Sm.  $\times 2360$ .
- 57 A spore with the extruded polar filament, showing also polar capsule with a part of the coiled filament in it. Sm.P.F.  $\times 2360$ .
- 58 A group of young sporoblasts. S.G.  $\times 1500$ .
- 59 A portion of the adipose tissue of a normal larva of *Culex pipiens*, showing three normal nuclei. S.G.  $\times 2360$ .
- 60 A portion of a heavily infected adipose cell with a hypertrophied nucleus. S.G.  $\times 2360$ .



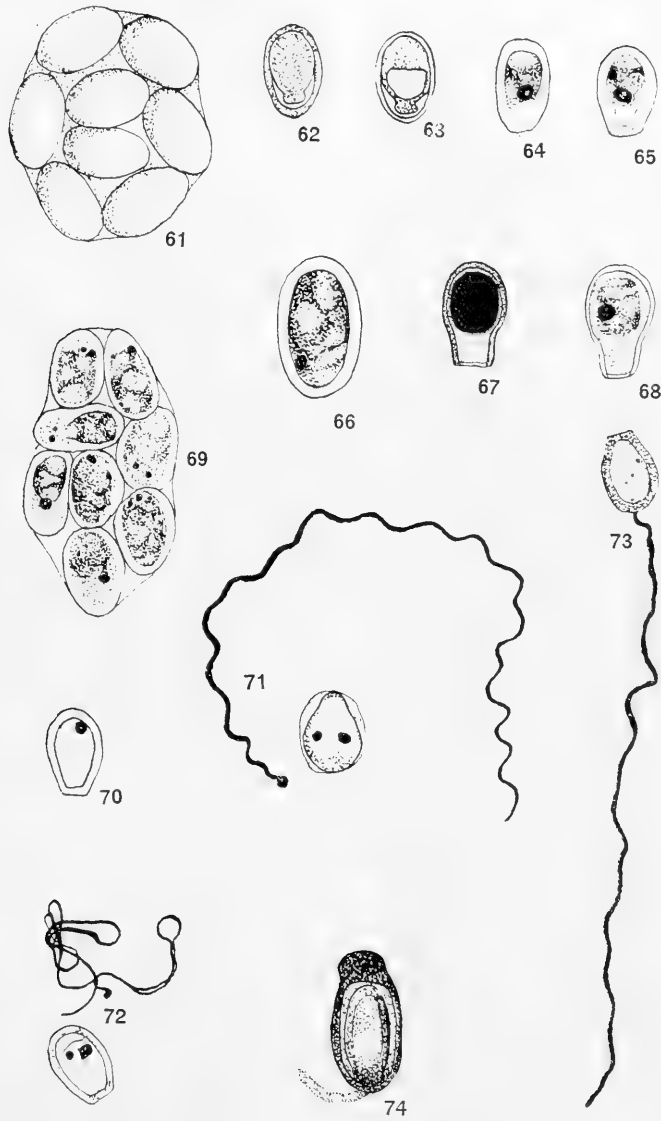


## PLATE 4

### EXPLANATION OF FIGURES

*Thelohania illinoisensis* nov. spec.

- 61 A fresh sporont, containing eight mature spores. Sm.  $\times$  2360.  
62 and 63 Isolated fresh spores. Sm.  $\times$  2360.  
64 and 65 Stained spores. Sm.G.  $\times$  2360.  
66 One of the young spores from the sporont shown in fig. 69, studied under higher magnification. Sm.G.  $\times$  3500.  
67 and 68 Two spores. Sm.H.  $\times$  3500.  
69 A sporont with young spores. Sm.G.  $\times$  2360.  
70 to 74 Spores with extruded polar filaments. Sm.P.F. Figs. 70 to 73,  $\times$  2360; fig. 74,  $\times$  3500, showing only the basal part of the filament.

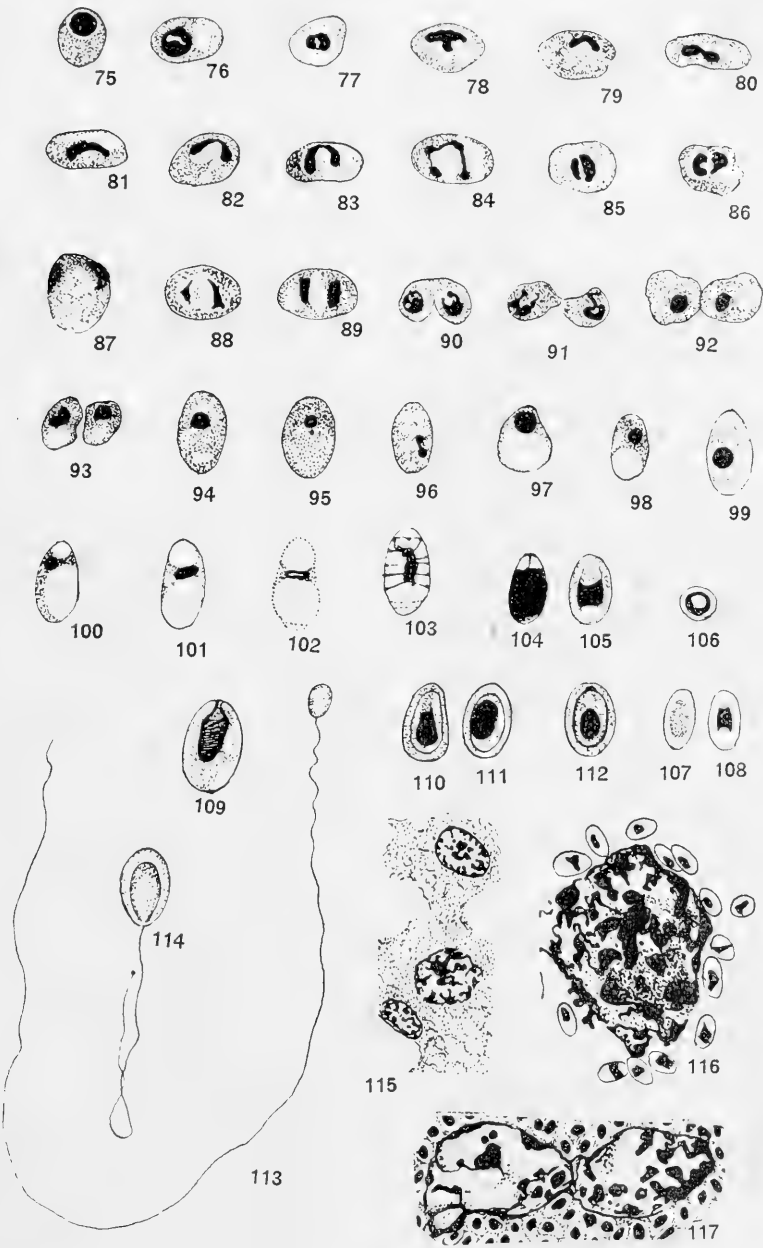


## PLATE 5

### EXPLANATION OF FIGURES

*Nosema beatis* nov. spec.

- 75 A schizont. S.G.  $\times$  2360.
- 76 to 93 Schizonts in various stages of binary fission. S.G.  $\times$  2360.
- 94 to 103 Development of spore. S.G.  $\times$  2360.
- 104 A young spore. S.H.  $\times$  2360.
- 105 A young spore. S.G.  $\times$  2360.
- 106 An optical section of a young spore. S.H.  $\times$  2360.
- 107 A mature spore. S.H.  $\times$  2360.
- 108 A mature spore. S.G.  $\times$  2360.
- 109 to 112 Spores. Sm.P.F.  $\times$  2360.
- 113 A spore with the extruded polar filament. Sm.P.F.  $\times$  1180.
- 114 A spore with partly extruded filament. Sm.P.F.  $\times$  2360.
- 115 A portion of the adipose tissue of a normal nymph. S.G.  $\times$  1500.
- 116 A hypertrophied nucleus of the heavily infected adipose cell of *Baetis* nymph. S.G.  $\times$  1500.
- 117 A hypertrophied nucleus undergoing amitosis. S.G.  $\times$  1500.



Resumen por el autor, Hirowo Ito.  
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Sobre la metamorfosis de los tubos de Malpigio de *Bombyx mori*, L.

1. La histólisis de la vejiga urinaria tiene lugar de tal modo que la membrana basal y la íntima se separan de las células epiteliales, con destrucción del citoplasma, y después los núcleos y dicha membrana son ingeridos por fagocitos, que los digieren. La íntima es expulsada de la cavidad durante la transformación en crisálida. La substancia muscular y los núcleos de las fibras musculares son destruídos completamente por los fagocitos. 2. La histólisis del tronco común tiene lugar de un modo semejante a la de la vejiga urinaria. La histogénesis del tallo imaginal común tiene lugar por una extensión gradual de las células imaginales situadas en la porción distal del tallo común de la larva. 3. La porción celómica del tubo de Malpigio pasa directamente desde el estado larvario al imaginal, si bien se disuelve el borde estriado y la membrana basal. El borde estriado imaginal y la membrana basal se forman por la secrección de las células epiteliales. 4. La porción de los tubos de Malpigio encerrada dentro de la pared del recto se destruye completamente, cuando tiene lugar la histólisis del aquel. Los núcleos se fragmentan en glóbulos que son ingeridos por los fagocitos. La membrana basal es atacada y destruída por fagocitos. 5. La función de los tubos de Malpigio parece cesar durante ciertos periodos de la metamorfosis.

Translation by José F. Nonidez  
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## ON THE METAMORPHOSIS OF THE MALPIGHIAN TUBES OF BOMBYX MORI L.

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FIFTEEN FIGURES

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### INTRODUCTION

The metamorphosis of the malpighian tubes of insects has been much studied by many authors: Karawaiew ('98), Anglas ('00), Pérez ('10), in Hymenoptera; Karawaiew ('99), Deegener ('00) in Coleoptera; Vaney ('02), Pérez ('01) in Diptera; Cholodkovsky ('87), Samson ('08), Hufnagel ('12), Ikeda ('13) in Lepidoptera. The results so far published are widely divergent, especially for Lepidoptera.

Cholodkovsky ('87) demonstrated the fact in *Tineola biselliella* that the malpighian tubes of the larva disappear gradually by histolysis, while the basal trunk elongates to form the imaginal tubes. A diametrically opposite view has been held by Samson ('08), who states that in *Heterogenea limacodes* that the malpighian tubes of the larva directly transform into the imaginal ones, although they undergo certain histological alterations.

Hufnagel ('12) investigated the metamorphosis of the tubes in *Hyponomeuta padella*, and came to the conclusion that one part of the larval tubes alone persists and differentiates into the

definitive organ, the remaining portion disappearing entirely. In *Bombyx mori* Ikeda ('13) has described a mode of development very similar to that described by Cholodkovsky.

The present study was undertaken to ascertain definitely in what manner the metamorphosis of the malpighian tubes takes place in the silkworm, and I was fortunate enough to obtain some results which have not hitherto been recorded regarding the following points: a) the histolysis of the urinary bladder; b) the histolysis and histogenesis of the common stem, and c) the histolysis of the portion of the malpighian tubes enclosed in the walls of the rectum, 'included portion.'

#### MATERIAL AND METHODS

The material for the present study was obtained from the univoltine race of *Bombyx mori*, known in Japan as 'Awojiku.' The larvae were fixed either in Perenyi's fluid or in sublimate alcohol warmed to about 80°C. For the pupae various fixing reagents were used. The best results were obtained with picrosulphuric acid and Perenyi's fluid. The material was embedded in paraffin. The sections were cut from 5 to 10 $\mu$  in thickness and were stained with Delafield's haematoxylin, picrofuchsin, and various other combinations of stains.

#### OBSERVATIONS

##### 1. *Malpighian tubes in larvae*

The malpighian tubes are situated near the posterior part of the body, opening into the ventrolateral side of the alimentary canal just at the junction of the small intestine and the colon. The tubes are six in number, three on each side. Two tubes first unite into one and then the third one fuses with the other two. The common stem of the three tubes is connected with the urinary bladder. This organ opens directly into the alimentary canal. Each malpighian tube runs at first forward as far as the anterior edge of the sixth or seventh segment, along the dorso-lateral side of the stomach. Then it turns on itself and goes backward a little beyond the colon, where it makes irregular



convolutions. Finally, the posterior portion of each tube enters the wall of the rectum and ends blindly after several convolutions. The outer side of the tubes is richly provided with tracheae.

For the sake of convenience, four regions may be distinguished in describing in some detail the histological structure of the tubes: *a)* the urinary bladder; *b)* the common stem; *c)* the coelomic portion which lies freely in the body-cavity, and *d)* the portion enclosed in the rectal walls, the 'included portion.'

*a. The urinary bladder.* The urinary bladder is ellipsoidal in form and measures about 0.95 mm. by 0.75 mm. in the fifth stage. Its epithelium is composed of large flat cells, the cell boundaries not always being clear. The cytoplasm, which stains a violet-pink with eosin, shows a reticular structure as in figure 3. The nuclei are round or oval, and differ greatly in size, according to the size of the cells. The nuclei are densely filled with coarse deeply staining chromatin granules. The inner wall of the epithelium is covered by a chitinous intima which is directly continuous with that of the intestine. There is no striated border, contrary to the description of Bordas ('11). The intima is fairly thick and does not stain, with either eosin or haematoxylin. The basement membrane is a fine transparent structureless limiting membrane. In some sections it is not readily detected. The circular muscles are strongly developed, and the striations of the muscles are clearly defined, as is shown in figure 3. There are some longitudinal muscles interior to the circular ones.

*b. The common stem.* The common stem is a narrow and short tube, measuring 0.52 mm. in length and 0.26 mm. in width. It does not differ in appearance from the urinary bladder as described above, excepting the smallness of the lumen, as is shown in figure 5. The numerous cells at the distal end of the common stem are very small and pressed closely together. These cells represent the imaginal ring (fig. 1, *ir*), although they never have a several layered appearance. The nuclei, which are round or oval, are densely chromatic. The chromatin granules are of the same color and size. During the larval stage the imaginal cells divide to elongate the coelomic portion.

*c. The coelomic portion.* The epithelium is composed of a single layer of two large polygonal cells. In cross-section, the cell boundaries are not well defined. The cytoplasm, which stains deeply with eosin, contains, near the striated border, many large vacuoles which are undoubtedly secretion products (fig. 4). Some faintly stained granular substance occurs in the vacuoles. The nuclei are irregularly branched, as shown in figure 1, and contain fine chromatin granules and nucleoli. The epithelium of this portion never has the intima, but instead is lined with a striated border, as has been observed by Schindler ('78), Bordas ('10, '11), Metalnikov ('08), and Veneziani ('03,

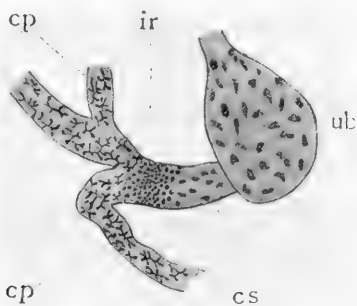


Fig. 1 Surface view of urinary bladder (*ub.*) and common stem (*cs.*) and coelomic portion (*cp.*) from a total preparation.  $\times 20$ .

'03 a, '04). The striated border is lightly stained and is marked by many very fine and closely set striations. The lumen differs in shape in different portions, and is filled with numerous granules and crystals of various shapes, such as cubical, octahedral, quadropyramidal, which are supposed to be made of a urate salt, so far as my examination goes. The epithelial cells have a very delicate transparent membrane which completely surrounds the tubes. Muscles are entirely lacking in this region.

*d. The included portion.* In a cross-section of the rectum (fig. 6), lying inside the circular muscles, is a nucleated peritoneum, which is a double layer, as has been described by Metalnikov ('08), Ishimori ('16). Though it is very difficult to make out the exact course of the malpighian tubes in the rectal wall, it can

be said that the more wavy it becomes, the larger folds in both the inner and outer spaces of the peritoneum. They thus surround almost completely the epithelium of the rectum. Both the cells and nuclei of the tubes in the rectal wall are very much smaller than those of the coelomic portion of the tubes. The epithelium is composed of very flat cells, especially in the outer space of the peritoneum. The cytoplasm is either homogeneous or granular in structure. The nuclei are also ramified, and densely chromatic, with small darkly stained chromatin granules. The striated border is not well developed in this region, but doubtless it is always present, at least in a feebly developed condition, even in the cases in which it is not visible. The lumen is narrower than that of the preceding region and contains some excreted granules which stain deeply with eosin.

## *2. Malpighian tubes in the prepupal stage*

The urinary bladder has become considerably reduced in diameter, measuring about 0.58 mm. by 0.25 mm. The cytoplasm has become vacuolated, and is gradually disappearing. The nuclei of the component cells have united to form a mass which stains deeply with haematoxylin. The intima is now separated from the cells and occurs shrunken in the lumen. The basement membrane is clearly defined, owing to the vacuolation of the cytoplasm, and is granular in structure. The muscles are also on the way to histolysis. The sarcolemma shrinks and is attacked by the phagocytes, and at the same time the striation disappears, as is the case with histolysis of the intestinal muscles. Finally, the muscular substance completely disappears, leaving behind some nuclei here and there.

In the common stem, just the same process of disintegration takes place as in the urinary bladder. The imaginal cells are greatly increased in number and size. The nucleus is round or ellipsoidal and contains many fine chromatin granules.

The coelomic portion comes in contact with the alimentary canal, which by this time has become very much flattened and contracted, owing to the digestion and excretion of the contents.

The distal end of the tubes has become narrow and shortly prior to pupation is detached from the rectum. The cytoplasm is homogeneous or very finely granular, and vacuoles no longer occur. The irregularly ramified nuclei are slightly shrunken and contain fine chromatin granules. The striated border is very faint and feebly developed, as is characteristic of the included portion. The lumen of the posterior portion is filled with homogeneous substance, while the anterior portion contains coarse granules. The basement membrane has the same character as in the preceding region.

The most remarkable histological change takes place in the included portion of the malpighian tubes, in connection with the histolysis of the rectum. The cytoplasm is granular and contains some small vacuoles. The ramified nuclei are fragmented into pieces, which form masses containing deeply staining chromatin in the form of a solid ball (fig. 7). Some massed nuclei have already been divided into globules. The lumen has completely disappeared as a result of the contraction of the epithelial cells. The phagocytes attack the basement membrane which sometimes is found already destroyed here and there, as is shown in figure 7. Such disintegration of the included portion of the malpighian tubes has not been observed by any previous authors.

### *3. Malpighian tubes in pupae*

In a pupa not older than twenty hours the cytoplasm of the urinary bladder and the common stem has completely disappeared. The massed nuclei are now fragmented into globules, which are gradually scattered in the body cavity, as is shown in figure 10. The intima is cast off from the lumen with the last ecdysis. There is no trace of the basement membrane, it being digested by the phagocytes. The muscles are also disorganizing by the attack of the phagocytes. The imaginal cells at the distal portion of the common stem increase in number by multiplication and gradually take the place occupied by the destroyed epithelial cells.

The coelomic portion of the tubes is detached from the alimentary canal and lies in convolutions among the fat-bodies.

The cytoplasm is granular and contains large vacuoles between the nuclei and the basement membrane. The nuclei are ramified as in the larval stage. The striated border disappears, probably at the last ecdysis. The lumen is conspicuously reduced in diameter, due to the contraction of the cells. The basement membrane comes in close contact with the periphery of the epithelium, as in the larval stage.

The included portion of the tube no longer retains its original shape, owing to the histolysis of the component cells. The fragmentation and dispersion of nuclear elements is more active, and the globules begin to migrate from the peripheral portion. Some globules are already being engulfed by the phagocytes (fig. 9).

In a pupa two to three days old, the massed nuclei of the urinary bladder and the common stem have entirely disintegrated into globules. The muscular substance has now completely disappeared, being digested by the phagocytes, and the muscle nuclei alone are still intact. A great many granular spheres are found around the muscle nuclei.

The coelomic portion now lies in the same position as in the preceding day. The cytoplasm is granular in the posterior portion and vacuolated in the anterior. The vacuoles gradually migrate toward the nucleus so as to come to lie between it and the surface of the cells lining the lumen. The ramified nuclei are central in position and contain many fine chromatin granules. Within the lumen are some granules and globules which are strongly eosinophilous. The basement membrane becomes gradually separated from the cells, as is shown in figure 8, and finally it is completely lifted up as a result of a considerable contraction of the cells. A great number of the phagocytes attack the basement membrane, as is shown in figure 11. This observation agrees well with that of Samson ('08), on the malpighian tubes of *Heterogenea limacodes*. Observing the destruction of the basement membrane, both Cholodkovsky ('87) and Ikeda ('13) came to the conclusion that the malpighian tubes of the larva completely disappear.

The nuclear globules in the included portion have completely disappeared, probably being absorbed by the phagocytes. There are many granular spheres surrounding the newly formed rectum. From this observation we may safely conclude that the malpighian tubes in the wall of the larval rectum are at first completely destroyed, and only secondarily do the phagocytes act in this destruction, as in the cases with the silk glands and the salivary glands. Since Samson ('08) has not studied the histolysis of the included portion, it is natural that he came to his conclusion.

In a pupa four days old, the muscle nuclei of the urinary bladder and the common stem have wholly disappeared through the action of the phagocytes and the imaginal cells have increased greatly in number. The coelomic portion lies embedded among the fat-bodies of the ventral region. The cytoplasm is homogeneous or granular, and stains deeply with eosin.

It is thought that the function of the malpighian tubes is arrested in four-day pupa. There are many chromatin granules in the ramified nuclei which stain somewhat faintly with haematoxylin. The lumen is, moreover, reduced in caliber and takes very irregular shape, varying greatly in different portions. The basement membrane now vanishes as a result of the activity of the phagocytes. The latter becomes enlarged; their contents assume a granular appearance.

In a pupa five to six days old, the imaginal cells gradually migrate to form the imaginal common stem which opens directly to the alimentary canal, as shown in figure 12, and the urinary bladder is not newly formed. The coelomic portion is in the same position as on the preceding day. The cytoplasm is granular and contains many vacuoles which are basal in position (fig. 13). The branches of the nuclei are extended and the chromatin granules become very distinct in outline. The lumen increases in caliber and contains some granules near the wall. These granules are the result of the secretory activity of the cells, and they indicate that the formation of the striated border has already set in. A very thin transparent basement membrane appears, which may be considered to be the secretion from the cells.

In a pupa seven to eight days old, the structures of the cells in the coelomic portion are almost the same as on the preceding day. The lumen is, however, increased in caliber and contains numerous granules (fig. 14).

In a pupa nine to ten days old, the formation of the common stem is already completed. The cytoplasm is granular and the nuclei are round or ellipsoidal and a thin new intima lies on the surface of the cells facing the lumen.

In the coelomic portion the cytoplasm contains very many large vacuoles which are situated between the nucleus and the striated border. The ramified nucleus is basal in position and contains some small, highly refractive nucleoli. The chromatin is in the form of small granules or rods, and appears to be arranged surrounding the nucleolus. The striated border is composed of many very fine and closely set striations. The lumen is very irregular in shape, especially in the anterior portion. The process of the excretion is very active, as indicated by the homogeneous or granular substance filling the lumen and staining light blue with haematoxylin. There are some vacuoles in the excreted substance of the posterior portion. The transparent basement membrane has increased in thickness.

In a pupa eleven to thirteen days old, the common stem and the coelomic portion remain in the same histological condition as described above. The excretory activity of the cells has increased, and the excreted substance stains deeply blue with haematoxylin as in the larval stage. It need hardly to be mentioned that the imago emerges on the fourteenth or fifteenth day after the pupation.

#### *4. Malpighian tubes in the imago*

In the imago the common stem opens directly at the junction of the stomach and the intestine, owing to the histolysis of the latter. The common stem is a short narrow tube and measures 0.61 mm. in length and 0.17 mm. in width. The epithelium is composed of large flat cells. The cytoplasm is granular and strongly eosinophilous. The nuclei are round or oval. The

intima is a thin chitinous membrane and is directly continuous with that of the intestine. The basement membrane is a transparent structureless membrane surrounding the tube.

The coelomic portion, which is provided with many fine tracheal branches, is convoluted in the ventral portion of the abdomen and ends blindly near the rectum. The distal end of the tube is very fine, and measures about 0.05 mm. in diameter. The epithelial cells are arranged in a somewhat peculiar fashion; they are too large to admit of their forming a smooth lining, the



Fig. 2 Surface view of coelomic portion from a total preparation.  $\times 50$ .

cell of one side projecting between two adjacent cells of the other, so that the entire tube looks knotty, as is shown in figure 2, and the individual cells are somewhat polygonal. The cytoplasm is granular or finely alveolar, and contains a great many large vacuoles. The nucleus is stained deeply with haematoxylin, and some nuclei have already undergone fragmentation. In such cases the chromatin comes together to form a mass. The striated border is definitely recognized by its fine and closely set striations, as is shown in figure 15. The lumen is irregular in section, and is filled with spheroidal blue granules and yellow



crystals, very probably a urate salt. The basement membrane is thin, structureless, and transparent.

#### SUMMARY

1. The histolysis of the urinary bladder is accomplished in such a way that the basement membrane and the intima are separated from the epithelial cells, with the destruction of the cytoplasm, and then both the nuclei and the basement membrane are ingested and digested by the phagocytes. The intima is cast off from the lumen at the pupation. The muscular substance and the muscle nuclei are completely destroyed by the phagocytes.

2. The histolysis of the common stem proceeds in a way similar to that of the urinary bladder. The histogenesis of the imaginal common stem takes place by the gradual extension of the imaginal cells which lie at the distal portion of the larval common stem.

3. The coelomic portion of the malpighian tube passes directly from the larval into the imaginal stage, though it undergoes the dissolution of the striated border and basement membrane. The imaginal striated border and basement membrane are formed by the secretion of the epithelial cells.

4. The portion of the malpighian tubes enclosed within the wall of the rectum is completely destroyed, along with the histolysis of the rectum. The nuclei break up into globules which are taken up by the phagocytes. The basement membrane is attacked and destroyed by the phagocytes.

5. The function of the malpighian tubes seems to cease during certain periods of metamorphosis.

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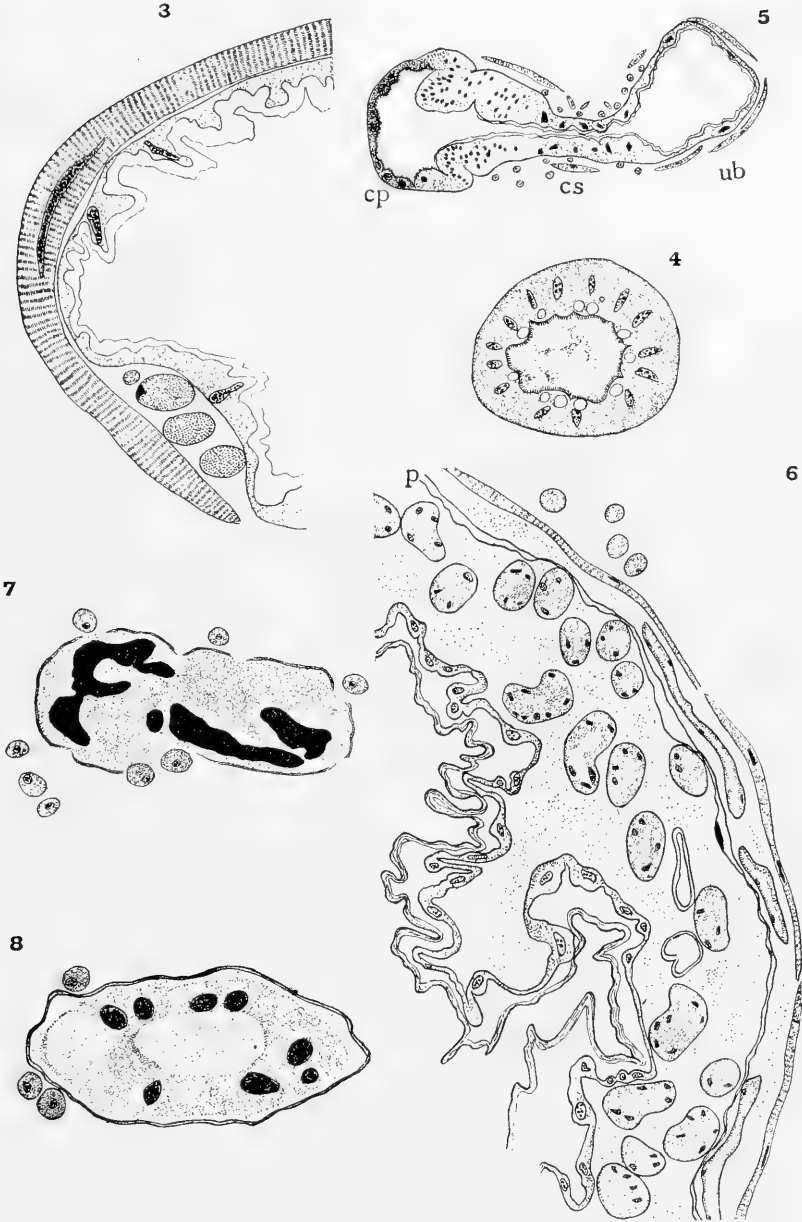
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## PLATES

## PLATE 1

### EXPLANATION OF FIGURES

- 3 Cross-section of the urinary bladder of a larva in the fifth stage.  $\times 400$ .
- 4 Cross-section of the coelomic portion of a larva in the fifth stage.  $\times 220$ .
- 5 Longitudinal section of the common stem (*cs.*) and the urinary bladder (*ub.*), showing the multiplication of the imaginal cells. *cp.*, coelomic portion.  $\times 90$ .
- 6 A portion of the rectum from a cross-section of a larva which has just begun to spin the cocoon, showing the included portion of the malpighian tubes. *p.*, peritoneum.  $\times 90$ .
- 7 A portion of the rectum from a cross-section of a larva on the day before pupation, showing the disintegration of the included portion of the tube.  $\times 400$ .
- 8 Cross-section of the coelomic portion of a pupa two days old, showing the separation of the basement membrane from the epithelial cells.  $\times 400$ .

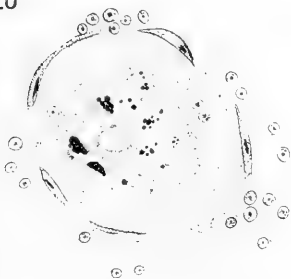


## PLATE 2

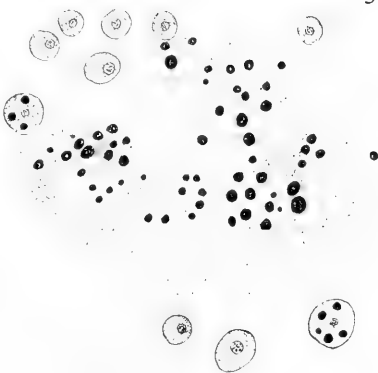
### EXPLANATION OF FIGURES

- 9 The disintegration of the included portion of a pupa just after pupation, showing the nuclei undergoing fragmentation into globules.  $\times 400$ .
- 10 Cross-section of the urinary bladder of a pupa just after pupation, showing the massed nuclei and the detritus of the muscles.  $\times 220$ .
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- 12 Cross-section of the coelomic portion and the imaginal ring, which is forming the imaginal common stem, of a pupa five days old.  $\times 160$ .
- 13 Cross-section of the coelomic portion of a pupa six days old.  $\times 220$ .
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- 15 Cross-section of the coelomic portion of an imago.  $\times 400$ .

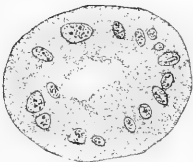
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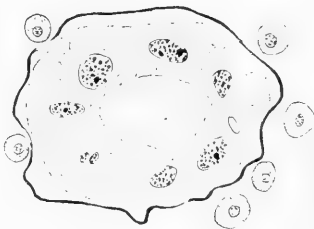
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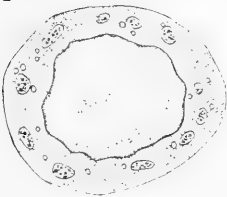
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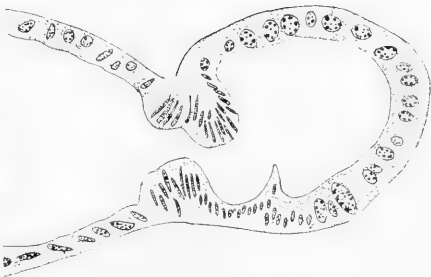
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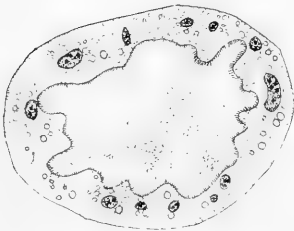
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12



15



Resumen por el autor, R. W. Shufeldt.  
Washington.

Observaciones sobre la región cervical de la columna vertebral  
de los Quelonios.

El presente trabajo considera brevemente las formas que los autores incluyen generalmente en el orden de los Quelonios—es decir, las tortugas terrestres y marinas, y los galápagos—siguiendo una discusión de las opiniones de diversos autores sobre el número de vértebras que existen en la región cervical de este grupo de animales, con especial mención de los trabajos de Günther, Claus, Owen, Hay, Boulenger, Reynolds y otros. El autor hace resaltar el hecho de que hasta el presente los autores admiten más o menos unánimemente la opinión de que hay ocho vértebras cervicales en el cuello de los Quelonios, y para demostrar el error de esta conclusión ha examinado la columna vertebral de unas doce especies diferentes y géneros de Quelonios de América y del Antiguo Continente, fotografiando la cadena de huesos cervicales de *Amyda* y otras formas.

En el caso de un individuo muy joven de *Amyda ferox* se practicaron cuidadosos cortes medianos de las vértebras del cuello, y estas secciones claramente demuestran que el hueso llamado hasta el presente “hueso odontoide” es en realidad los vestigios de la vértebra axis del cuello de la tortuga; en *Amyda* este elemento posee un proceso odontoide rudimentario. Este proceso ha sido representado en las láminas, las cuales representan fotografías de las partes en cuestión.

Translation by José F. Nonidez  
Cornell Medical College, New York



## OBSERVATIONS ON THE CERVICAL REGION OF THE SPINE IN CHELONIANS

R. W. SHUFELDT

FIVE FIGURES

In passing in review some of the literature on the osteology of the chelonians, it appears that not all of the contributors to it are in full agreement with respect to the number and characters of the cervical vertebrae. Intelligently to discuss these differences of opinion, and the figures that have been published to illustrate them, it will, first of all, be necessary to define where the cervical division of the spine in a chelonian begins and terminates. As to its beginning, there is no question, for the first vertebra of the chain is the atlas, as in all the Vertebrata, and it articulates with the occipital bone of the cranium by a single, median condyle.

Günther ('86, pp. 445 and 449) very clearly expresses his views on this subject. In defining the order Chelonia, he states: "Cervical and dorsal vertebrae not numerous. The dorsal vertebrae and expanded ribs (with the exception of Sphargis) are united into a carapace, the elements of which are immovable, and which is completed ventrally by a number of dermal bones, a true sternum being absent and replaced by a plastron." According to this very clear statement, the anterior dorsal vertebra is the first immovable one, which, by the aid of its pair of ribs, enters into the formation of the extreme forepart of the carapace. All the vertebrae between this one and the skull are free and ribless, and together constitute the true cervical vertebrae. Further on in this article (p. 449), referring to the spinal column in the Chelonia, he says: "There are always 8 cervical, 12 dorsal, and 2 sacral vertebrae." According to this, then, it is very definitely fixed as to which vertebrae are to be considered cervicals and which dorsals, and the exact number of each division of the spine in all existing chelonians.

Claus ('85), in defining the spinal formulae in the *Chelonia*, is somewhat at variance with Günther. He states that "unlike the middle (thoracic) region of the vertebral column, the vertebrae of which are firmly connected with the dorsal shield, the cervical and caudal vertebrae are always movable upon one another. The cervical region is exceedingly flexible, and can be more or less completely retracted within the shell; it consists of eight long vertebrae, which are without ribs. The ten rib-bearing vertebrae are followed by two or three sacral vertebrae, which project beneath the carapace, and by a considerable number of very movable caudal vertebrae" (p. 227).

This sustains Günther's definition of the cervical and dorsal vertebrae with respect to their kind, but not as to their number, for, as pointed out above, the latter authority states that without exception all chelonians possess twelve dorsals and but two sacrals, while Claus says very distinctly that they have but ten dorsals and "two or three sacrals."

Claus also figures the "skeleton of *Cistudo (Emys) europea*," in which there are shown, according to his own definition of them, nine cervical vertebrae; eight thoracic or dorsal vertebrae; three sacrals, and some twenty-five caudal vertebrae. Thus, at the very start, we meet two statements quite at variance with each other with respect to the number of vertebrae in the several divisions of the spine in existing chelonians.

Sir Richard Owen has also given us a figure of the "skeleton of *Emys europea*" ('66, p. 60, fig. 51), which has been made to possess but seven cervical vertebrae and, apparently, not more than eight dorsals, while in the text he states that "all the eight cervical vertebrae, fig. 51, E, are free, movable, and ribless."<sup>1</sup>

Owen also claims that "three vertebrae form the sacrum" in the *Chelonia* and mentions no exceptions to it.

<sup>1</sup> In completing this sentence, Owen states that "the fourth of these vertebrae has a much elongated centrum, which is convex at both ends; the eighth is short and broad, with the anterior surface of the body divided into two transversely elongated convexities, and the posterior part of the body forming a single convex surface, divided into two lateral facets; the under part of the centrum is carinate; the neural arch, which is ankylosed to the centrum, is short, broad, obtuse, and over-arched by the broad expanded nuchal plate."

Before examining some turtle skeletons at hand, it may be as well to quote the opinions of more recent authorities on chelonian osteology; we turn to the work of one of the best-known writers on this subject of chelonian osteology—Doctor Hay, who states ('02, p. 4) that "We may now examine the vertebrae in front of and behind the dorsals. In all of the turtles there are normally 18 presacral vertebrae, of which 8 belong to the neck. The more anterior and the more posterior cervicals are shorter. The neck as a whole is about as long as the dorsal series of vertebrae. The first is composite, consisting of four distinct pieces. On each side is a neural arch, aiding in forming the neural canal. Below, these abut on a median piece, the hypocentrum. These three bones unite in forming a concavity, into which fits the ball-like occipital condyle. Behind the arches and the hypocentrum is the odontoid process, the proper centrum of the first cervical. Behind, this articulates with the centrum of the second cervical, but does not become ankylosed with it."

Boulenger says ('89, p. 15): "The cervical vertebrae, which number eight as in all Chelonia, present this peculiarity that their centra exhibit the four modes of articulation, some being concavo-convex, others convexo-concave, others biconvex, others biconcave. A single exception is known, *Pyxis*, in which they are all procœlus in the specimen examined by Vaillant, as well as in the one in the British Museum." Boulenger appears to have examined the vertebral column of a number of the soft-shelled turtles, but on the whole somewhat superficially.

Indeed, it would appear that all of the recent writers on the subject state that the chelonians have eight cervical vertebrae in their spinal columns. As a final authority we may cite Reynolds, who, in describing the cervical vertebrae of a turtle, says ('97, p. 219): "These are eight in number, and are chiefly remarkable for the variety of articulating surfaces which their centra present, and for their mobility upon one another."

A number of our text-books on zoology, now in use in schools and colleges, state that all chelonians possess eight cervical vertebrae, but it would appear that the authors of such volumes have never personally verified this statement, rather obtaining it from the standard works on the subject of an earlier date.

We are now in a position to examine some chelonian material and compare what it presents with respect to the vertebrae and to contrast the results with the statements of the various authorities introduced in the foregoing paragraphs.

In my private collection there are perfect skeletons of the following turtles and tortoises:

1. Gopher tortoise (juv.) *Testudo polyphemus*.
2. Box tortoise (numerous skeletons) *Terepene carolina*.
3. Musk tortoise, *Aromochelys odoratus*.
4. Mud turtle, *Cinosternum pennsylvanicum*.
5. Painted turtle, *Chrysemys picta*.
6. Bell's terrapin, *Chrysemys belli*.
7. Cumberland terrapin (juv.) *Chrysemys elegans*.
8. Yellow-bellied terrapin (juv.) *Chrysemys scabra*.
9. Lesueur's terrapin, *Malacoclemmys lesueurii*.
10. Spotted turtle, *Chelopus guttatus*.
11. Snapping turtle, *Chelydra serpentina*.
12. Soft-shelled turtle, *Amyda ferox*.

In addition to the above skeletons, I also have before me the material given below, for the loan of which I am indebted to the United States National Museum, to which institution it belongs.

1. *Testudo ehippium* (Duncan Island, Galapagos). No. 59867, cervical vertebrae.
2. Leading cervical vertebrae of a crocodile from Batavia, Java (May, 1909)—species? Incomplete; free 'odontoid' of second vertebrae missing.
3. Hawksbill turtle, *Eretmochelys imbricata*. Key West, Florida. No. 59866. Cervical vertebrae; hyoid; trachea; caudal vertebrae; adult.
4. *Amyda ferox*, adult. Florida. Cervical and caudal vertebrae; pectoral arch. No. 60534.
5. *Testudo* sp? No. 61059. Vertebrae and other bones. National Zoölogical Park, July, 1918.
6. *Amyda cartilagina*, adult. No. 029550, Depok, Java. Spinal column complete.

Omitting the second or axis vertebra, which will be taken up further on, it may be noted that in the chain of cervical vertebrae

of both *Testudo ephippium* (fig. 1) and of *Eretmochelys imbricata* there are eight bones; but whether this eighth one is followed by a true dorsal vertebra I am unable to say, for the reason that I know nothing of the preparation of this material, nor have I seen, in either case, the rest of the vertebral column.

Still omitting the axis vertebra, the seventh of either of these series is peculiarly formed, its neural spine, situated on the forepart of the bone, being enormously developed and elevated far above the centrum, being broad and spreading, with its superior aspect converted into a great, cup-like concavity, the posterolateral projections of which are the postzygapophyses. This peculiar formation is most highly developed in the great tortoise of the Galapagos, though it is present, too, in the hawksbill, where it is so extended as to include most of the modified neural spine of the vertebra next beyond it. In the latter, all the cervicals are considerably shorter than in the former species, where they are much elongated and present entirely different characters.

In neither of these gigantic species do we find the inferomedian part of the atlas vertebra coossified with the rest of that bone, and especially is this evident in the hawksbill.

Both species present a foraminal perforation at the middle of the base of the 'cup,' which, in many vertebrates, admits the anterior end of the odontoid process of the second or axis vertebra.

Throughout the literature of the osteology of the chelonians, this second vertebra has been simply nominated the 'odontoid bone,' and no writer heretofore has considered it in the light of one of the cervical vertebrae. As a matter of fact, it is the centrum of the axis, with the neural arch and the apophysis usually found on this bone, aborted. The true odontoid process is still to be found well developed at its usual site in some existing turtles, and particularly in those of the genus *Amyda* (figs. 3, 4, and 5). Indeed, in *A. cartilaginea*, the axis or second cervical vertebra not only has a well-pronounced odontoid process, but the anterior face of the centrum presents three facets for articulation with the atlas—an inferomedian one and an upper one upon either side above it. Inferiorly, the centrum is produced

as a thickened haemal spine. Posteriorly, it offers the usual cup-like articulation of the third cervical vertebra.

This to some extent aborted or vestigial axis vertebra lacks both pre- and postzygapophyses; while, when the cervical vertebrae are duly articulated as in life, the prezygapophyses of the third vertebra extend forward, to articulate with the postzygapophyses of the atlas, which are elongated, and extend backward and outward for that purpose, the circular articular facets being, on their mesial aspects, somewhat in advance of the end of the process on either side.

These and other conditions are distinctly foreshadowed through what occurs in the very young and subadult specimens of *Amyda ferox*. Through the kindness of Mr. F. W. Walker, of Orlando, Florida, and Mr. Edward S. Schmid, of Washington, I have been abundantly supplied with such material, and, further, I am greatly indebted to Dr. Charles Judson Herrick, of the University of Chicago, for having had made for me, by his assistant, Miss Jeannette Obenchain, nearly forty microscopic slides of the cervical region of the vertebrae column in young *Amyda ferox*. These present the morphology of the cervical vertebrae at these young stages in the soft-shelled turtles. The series well demonstrates the several centers of ossification of the atlas; the fact that the centrum of the axis is formed in the same line and in the same manner as the centra of the other cervicals of the column, and clearly shows its articulation with the atlas anteriorly and the third cervical which immediately follows it.

Counting, then, the first two bones in the neck of the turtles of the genus *Amyda* as the atlas and axis, the third cervical, or the bone next in order, is narrow and much elongated—characters that gradually change as we proceed backward through the series to include the eighth from the skull (figs. 3 to 5). They become progressively shorter and broader, with forms that are well shown in the accompanying plates.

It was Huxley's opinion that "In a great many Vertebrata, the first and second cervical or atlas and axis vertebrae undergo a singular change; the central ossification of the body of the atlas not coalescing with its lateral and inferior ossifications, but

either persisting as a distinct os odontoideum, or anchylosing with the body of the axis, and becoming the so-called odontoid process of this vertebra" ('72, p. 18).

We have here a declaration which it is evident sustains the fact that in *Amyda*, and doubtless in other turtles, the so-called odontoid bone is in reality the second cervical or axis vertebra, inasmuch as we find it supports an odontoid process.

Professor Huxley makes no mention, in the work cited, of the fact that the postzygapophyses of the atlas extend backward to articulate with the prezygapophyses of the third cervical vertebra, and in doing so pass over the axis vertebra as pointed out above. There may be exceptions to this among the Chelonia, but if so, the species are not recalled at this writing. The fact that the axis has become largely vestigial in character is doubtless based in the manner of use of the neck in a turtle. The rotary movements of the skull involving the axis are far more limited than in other Vertebrata; while the ability to draw the head back into the carapace and to thrust it suddenly forward, as most turtles do, demands a peculiar action in one plane—hence a certain abrogation of function in the distal end of the cervical vertebrae; elongation of the median ones, and, again, a shortening of those as the carapace is approached. We find the same condition of things among such birds as bitterns, herons, and other waders.

All chelonians, irrespective of genus or family, in so far as I have examined them, present a more or less sudden and peculiar change of form in the cervical vertebra marked 9 in these plates. This is particularly true in the case of the soft-shelled forms of the genus *Amyda*. Here, when viewed directly from above, it is almost square in outline, being rather wider posteriorly than it is in front. Both neural and haemal spines are entirely absent, while the prezygapophyses are small, subcircular in outline, and to some extent tilted backward. The extension backward of their mesial margins are sharp, and by meeting in the middle line, they form two sides of an equilateral triangle, the base of which is an imaginary line joining the prezygapophyses anteriorly. Below this imaginary line in front, situated side by side,

and standing apart by quite an interval, there are two conspicuous articular facets, each being semiellipsoidal in form, with its major axis placed transversely. To either outer side of one of them, there is a bluntly rounded process, each somewhat curved toward the median plane of the bone. The neural canal is large, short, and cylindrical in form. The broad centrum, of triangular outline posteriorly, is but a mere thin plate, smooth, and moderately concaved on its under side. Still more posteriorly are the enormously developed postzygapophyses, divided by a shallow, triangular median notch behind. Each has a rounded outline, the general surface of the two above being smooth and uniformly convex. Upon the inferior aspect each is markedly convex from before backward, the extensive outer surface of either one being smooth and articular in character. All of these points are well shown in the several plates in which this vertebra appears.

Counting the axis as the second vertebra from the skull in the cervical series, as shown in the plates (figs. 3, 4, and 5), we next come to the one marked *10* in the figures. To this vertebra I have given very careful study in all the material at my hand, including typical land tortoises from various parts of the world, pond turtles, the great marine forms, and the soft-shelled types.

In the land tortoises, such as *Testudo* and *Terepene*, and many others wherein the carapace of the shell is entire, with all of its component parts solidly coossified together, and not a semblance of an hiatus among any of them, this vertebra, while it presents a number of morphological characters referable to the true cervical vertebra next in advance to it in the living animal, must nevertheless be considered as the first dorsal vertebra in such chelonians as I have thus far examined. In the various species of *Amyda*—and especially in such forms as *Amyda cartilaginea*—at least two-thirds of its characters pertain to a cervical vertebra at the termination of the series. Its centrum projects considerably beyond the postzygapophyses above it (figs. 3 to 10), and each of its postero-external angles presents a small, roughened surface, that in life is feebly coössi-



fied with the ventral surface of the anterior margin of the first neural plate of the carapace. Upon either side it extends backward and outward as a slender rib that distinctly fuses, through feeble coossification, with the anterior margin of the first true dorsal rib just beyond its head. The forepart of this vertebra no. 10—its dorsal aspect—simply rests, mesially, against the ventral aspect of the nuchal plate of the carapace, and at this point it is articulated with vertebra no. 9, as shown in the accompanying figures on the plates. Our United States species of the soft-shelled turtles present identically the same arrangement and morphology as this, and it is fair to presume that all other true species of the genus *Amyda* do the same.

Coming next to the snapping turtles (*Chelydra*), this vertebra no. 10 has assumed a form that distinctly stamps it as a true dorsal vertebra. It possesses a conspicuous neural spine that barely reaches the articulation of the nuchal plate of the carapace, anteriorly; it has a small pair of ribs of its own, either one of which, for its outer two-thirds, fuses, in adult individuals, with the anterior margin of the true dorsal rib of its own side, as far as the middle point of the latter. Posteriorly, this vertebra presents a demifacet upon either side of its centrum for articulation with the anterior part of the head of the first true dorsal rib next to it upon either hand. In old specimens of *Chelydra serpentina* all of these osseous parts become thoroughly coossified and form one solid piece. When this happens, the vertebra in question is entirely and indistinguishably incorporated with the animal's shell. Between *Amyda* and *Chelydra*, then, we note a transitional stage, with respect to this tenth vertebra—a stage where it is passing from a cervical to a typical dorsal one of the series. While in all existing species known to me at this time, the ribs are of a more or less rudimentary type, it is quite possible to conceive that in the progenitors of the line of chelonians, from which the existing species of *Amyda* have arisen, this particular vertebra was a true cervical one; that is to say, it possessed no ribs and was in no way coossified with the carapace of the shell in adult individuals.

Doubtless, among the Reptilia generally, we will meet with some very interesting variations of the conditions just described, and they are deserving of far more extensive attention at the hands of comparative morphologists than they have received up to the present time.

#### CONCLUSIONS

That the element heretofore generally considered as the 'odontoid bone' in the cervical series of vertebrae in the chelonians is, in fact, the centrum of the axis or second vertebra of the neck—the true odontoid process being present in such species as constitute the genus *Amyda* and possibly others. That this second or axis vertebra has, for some reason or other, lost its arch, and in some forms only, as in *Chelydra*, is there any indication of a haemal spine being present. As in other vertebrates, this to some extent aborted second cervical vertebra, articulates anteriorly with the atlas, and posteriorly with the third cervical, and is just as much entitled to be considered the axis vertebra as are various semiaborted bones in the skeletons of other vertebrates so considered. For example, to illustrate this point we may select, from a long list of others, the fibula in certain Cervidae, as the red deer (*C. elaphus*), wherein it has become reduced, distally, to a mere nodule of bone, and, proximally, to a rudimentary osseous style—the two never being united by bone. Nevertheless, anatomists consider these two osseous rudimentary elements in the leg of a deer as being the fibula, and so name and describe it.

Having proved, then, that the so-called 'odontoid bone' is, in reality, the axis vertebra in the chelonian skeleton, we find that there are nine vertebrae in the cervical series of the skeleton in the chelonians instead of eight, as comparative anatomists have heretofore claimed to be the case.

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## EXPLANATION OF THE PLATES

All figures in the plates are from photographs by the author; direct from the specimens and reduced about one-third.

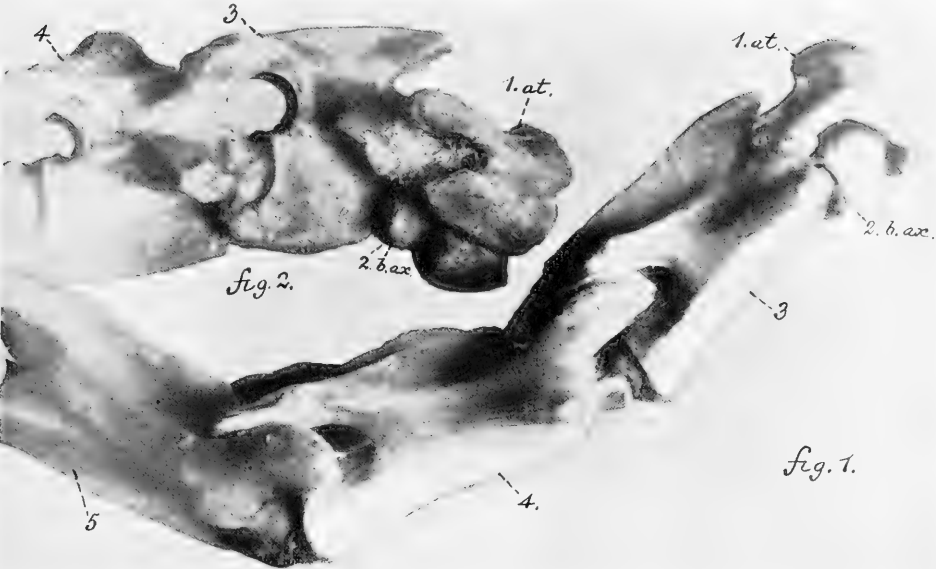
### PLATE 1

#### EXPLANATION OF FIGURES

1 Right lateral view of the first five vertebrae of the neck (cervicals) from an adult specimen of *Testudo ephippium*. 1, *at.*, atlas; 2, *b.ax.*, body of the axis or second cervical vertebra; 3, third cervical; 4, fourth cervical, and 5, fifth cervical vertebrae. No. 59867, Coll. U. S. Nat. Mus.

2 Right lateral view of the first four vertebrae of the neck (cervicals) from an adult specimen of the hawksbill turtle (*Eretmochelys imbricata*). Lettering as in figure 1. No. 59866, Coll. U. S. Nat. Mus.

3 Right lateral view of the leading ten free vertebrae in the neck of an adult specimen of *Amyda cartilaginea* (1 to 10). *o*, the odontoid process of the second or axis vertebra. No. 029550, Coll. U. S. Nat. Mus.

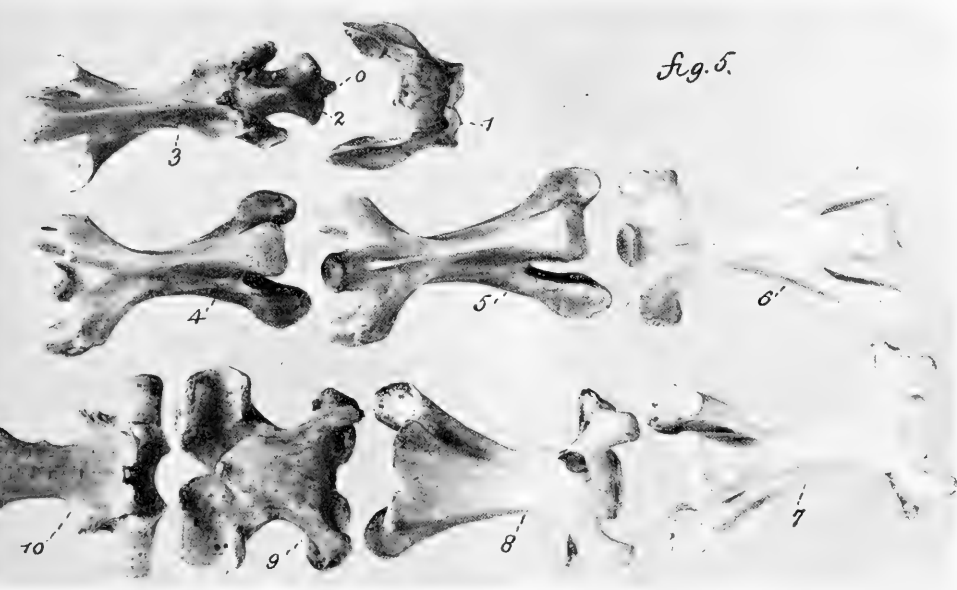


## PLATE 2

### EXPLANATION OF FIGURES

4 Same vertebrae as are shown in figure 3. 1, 2, and 3 seen on semioblique right lateral view; 4 to 10 viewed nearly on direct superior or dorsal view. Lettering as in figure 3.

5 Same vertebrae as are shown in figures 3 and 4. All seen upon direct ventral or inferior view. Lettering as in figures 3 and 4.



Resumen por el autor, Augustus G. Pohlman.  
Saint Louis University.

La posición e interpretación funcional de los ligamentos elásticos  
de la región del oído medio de Gallus.

El presente trabajo trata de la anatomía aplicada de la región del oído medio, basada en Gallus. El autor describe con detalle la posición de los ligamentos elásticos y su relación con el aparato transmisor del sonido, cuando se consideran en conjunto con un sistema de articulaciones que se encuentra en el complejo columelar. Mientras que el músculo stapedio y el tensor del tímpano del mamífero se consideran como sinergistas, el músculo tensor del tímpano del gallo parece desempeñar la función de ambos músculos citados, y su existencia puede atribuirse a un doble desplazamiento en el mamífero, que tiene lugar en relación con la cadena osicular, mientras que en las aves existe un solo desplazamiento. El autor mantiene que el músculo y el aparato columelar están adaptados a una topografía variable de la membrana timpánica, debida a las presiones relativas del aire en el oído externo y medio.

Los ajustes no están probablemente asociados directamente con la agudeza del oído y la tensión del tímpano es probablemente un resultado y no una causa. El autor compara las necesidades mecánicas en las aves y mamíferos, y cree que las vibraciones moleculares del aire que llegan al tímpano son proyectadas sobre la ventana vestibular, y que las necesidades generales, según se establecen primeramente en los anuros terrestres, persisten en todas las formas superiores de los vertebrados terrestres.

Translation by José F. Nonidez  
Cornell Medical College, New York



## THE POSITION AND FUNCTIONAL INTERPRETATION OF THE ELASTIC LIGAMENTS IN THE MIDDLE-EAR REGION OF GALLUS

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TWELVE FIGURES

### HISTORICAL

Little work has been done on the applied anatomy of the middle-ear region in birds, and it was thought that a detailed study of the structures and mechanics of this region might lead to a better understanding of its function. The great difficulty in determining the function of the middle ear in mammals comes about through inability to find a suitable form in which the definite relation of structure to function may be experimentally established.

It may be well to state that Denker's ('07) excellent monograph on the inner-ear region of the parrot, undertaken with a somewhat similar point in mind, was not productive of interpretation because of the dissimilarity of the structures in birds and mammals. However, this does not militate against a comparative consideration of the middle-ear mechanics, because birds hear as acutely as or more acutely than mammals; the sound transmitting apparatus is of more simple construction and open to as little variation as the ossicular chain in mammals, and, finally, because of the differences in this region in birds and mammals, some conclusions may be attained bearing on the variable factor which calls for muscular adjustment in both forms. If one must go as far back as the amphibians for an explanation of the ear-cough reflex and class it as a phylogenetic reflex associated with the respiratory function of the middle ear, it is probably safe to assume that the general functional

requirements of this region are about the same in all forms. Here again we find inadequate explanation of the significance of the opercular muscle and the functional interpretation of a movable skeletal element applied to the perilymphatic space of the anurans.

The bibliography on the function of the middle-ear region in birds is not surprisingly large. Krause's ('01) monograph on the form of the columella and the relation of its form to acuteness of hearing is not productive of definite results or convictions. He concludes that, while the form of the sound-transmitting apparatus has something to do with the acuteness of hearing, much must be referred to the development of the end organs and to the central nervous system. His excellent figures demonstrate that the columella in birds is not subject to wide variations in form. It is impossible in the limited scope of this paper to go into any great amount of comparative detail, and the discussion will be limited practically to the conditions as found in the chicken. A general description of the relations of the structures in the middle-ear region of birds is, however, essential because only one article presents anything like a comprehensive account and none is adequately illustrated.

The best general description of the middle-ear region in birds is to be found in the account of Breuer ('08), whose abstracts and deductions read about as follows:

The drum membrane of birds presents a marked inversion in its relations to that found in mammals. The drum membrane in mammals is drawn inward toward the *cavum tympani*, while in birds, it is pushed outward. The *manubrium mallei* extends from the upper border of the drum toward its mid-area (in man, from above ventrally); in birds, the corresponding cartilage apparatus extends downward and backward from the central area of the drum toward its periphery. In mammals, the uppermost portion of the drum is flaccid; in birds, the relaxed portion is below. If one imagine a human ear drum turned through  $180^\circ$  on an axis represented by a diagonal line drawn across its surface from the postero-superior to the antero-inferior margin the resulting relations would represent roughly the position of the structures found in birds.

The drum membrane in birds, including the entire sound-transmitting apparatus, has but a single muscle attached to it and corresponding with the above mentioned inverted relations, the *M. tensor tympani*

Breschet) is not an intrinsic muscle of the tympanum, but arises from and is applied to the outer surface of the skull.

Breschet ('36) describes the muscle in the following manner: The malleus (by which Breschet means the cartilaginous portion of the columella attached to the drum) has strictly speaking a single muscle, *M. externus* (laxator) memb. tympani. This muscle attaches to the malleus at the union of the body of the bone with the stalk of the columella. Its tendon, at first fused to the drum, loses its connection at the drum margin; passes into a bony canal; emerges from the tympanum; becomes fleshy; and attaches to the lower border of this cavity. It is here covered by the *Nn. glossopharyngeus* and *vagus*. (There is undoubtedly some error in the translation of the French into German. The text itself was not available.)

The inner muscle of the malleus (*M. tensor tympani*) is present only as a rudiment. It is a delicate strand of connective tissue, particularly prominent in the turkey where it is well separated from the drum membrane. I have investigated it in other forms and have found it constant and united with the drum as a glistening pearly streak. This attaches to the malleus and runs forward and medialward; follows the general direction of the Eustachian tube and merges with the fibrocartilaginous lining of the tube itself. The action of the muscle is naturally a very limited one because it can propagate to the drum only those movements imparted to it by the motion of the tubal walls to which stout muscles are attached. It is hardly necessary to state that, if one pulls upon this strand or upon the tubal walls to which it is attached, the drum membrane becomes more tense. Traction upon the tendon of the *M. externus* causes a relaxation of the drum; an experiment which demonstrates the respective function of each.

Gadow ('93) describes the muscle in the following manner: The muscle is relatively stout; arises by fleshy fibres from the lower surface of the *os occipitalis basilaris*; and passes through a large opening into the *cavum tympani*. The muscle renders the drum more tense and draws it outward. The muscle corresponds to the *M. tensor tympani* of the mammals, and is innervated by a fine twig of the *ramus III* of the *N. trigeminus*. A *M. stapedius*, innervated by the *N. facialis*, is wanting in birds.

Gaupp ('98) remarks: It is difficult to believe that this description is correct. It is in direct opposition to what is known of the middle ear muscle of the *Sauropsidans* and above all is contrary to Killian's positive statements. Furthermore, the innervation of a muscle attached to the posterior border of the drum through the *N. trigeminus* would be a remarkable thing. The notations of Killian, ('90) who undoubtedly worked on this same muscle, give its nerve supply through a separate branch of the *N. facialis*. Killian found this to be true in duck, goose, and chicken, and describes the embryonic attachments of the muscle first to the extrastapedial and later into the margin of the drum membrane proper.

According to Geoffry Smith ('04): The columella is provided with a single muscle. The *M. tensor tympani*, which attaches to the infra-stapedial and the drum margin between the infra- and extrastapedial cartilages. The muscle passes out of the ear through a wide opening, close to the foramen stylo-mastoideum, bends to the posterior surface of the skull, and attaches to the basioccipitale in a gentle depression which extends almost as far as the condylus occipitalis.

I will confine the description of this questioned muscle for the most part to its relations in the pigeon and chick which I have compared with a few other forms. Inspection of the figures given by Breschet shows that here, as well as in the relations of the organ of hearing itself, a very great similarity exists in the various classes of birds.

The sound-transmitting apparatus, which lies between the drum and the labyrinth, is formed by the columella. This resembles a mammalian stapes provided with a long stalk and attached laterally to a cartilaginous apparatus which has been homologized in part with the malleus and incus. This cartilage may be called the cartilage-head of the columella (extra-stapedial of Huxley). Its figure is that of an obtuse angle triangle with one point and the baseline attached to the drum. The latter is placed radially in the posterior-inferior quadrant of the drum membrane which is thickened between the head of the columella and the drum margin by tendinous fibres. Cartilaginous processes arise from either side of this structure posteriorly and inferiorly, somewhat raised on the inner surface of the drum and attached to the borders of this membrane. Just as the struts support the canvas of a tent, so the head of the columella together with its cartilaginous processes press the drum outward, and indeed even after the columellar stalk has been severed, the elasticity of the cartilage-head and its processes is sufficient to maintain the drum convexity.

If one divides the drum by two diagonal lines, four quadrants result which are somewhat different in structure. The ventral upper quadrant forms a regularly stretched conical mantle; the lower is flaccid; while the posterior quadrant is thickened by tendinous fibres particularly near its margin. The *M. tensor tympani* arises, as described by Gadow, from the outer surface of the skull close to the condyle; is directed horizontally forward and lateralward toward the bony border attaching the drum membrane. The *Nn. glossopharyngeus* and *vagus* emerge from the skull at its lower border. The muscle passes through an opening in the bony border of the external auditory canal and sends its tendinous fibres to attach to the drum margin medially below the columellar head. It therefore never enters the tympanic cavity as described by Breschet. The tendon breaks up in a sort of *pes anserinus* which unites with the drum; the upper portion however coming from below and behind unites to the manubrium-like process of the head of the columella.

The muscle is innervated by the *N. facialis* and may be stimulated with ease and certainty through the trunk of this nerve. This may be accomplished through a dissection exposing the semicircular canals and

by introducing a protected electrode into the foramen communicans just posterior to posterior canal. Here the electrode is in contact with the N. facialis and the other electrode may be placed on an indifferent part of the bird's body. When the circuit is closed, the head of the columella is drawn backward and downward; the upper and anterior quadrants of the drum are rendered tense and the concavity of the conical surface is decreased. The lower quadrant is rendered more tense from before backward but because the head of the columella slips downward, it is relaxed in this direction. (This makes it simple to understand why Breschet called the muscle a *laxator memb. tympani* because one would assume, unless it was demonstrated to him, that a M. tensor tympani would not under any circumstances be an extrinsic muscle of the middle ear.)

The columella returns to its original position when the stimulation of the N. facialis ceases. This comes about through the elasticity of the cartilaginous processes which were bent through muscular contraction. Particularly the lower process, which Breschet homologizes with the proc. gracil. mallei, seems to react because of its spring-like form; while the upper cartilage process seems to prevent too great a backward displacement by pressing against the drum border. When the head of the columella is pulled backward, the plate closing the oval window must also be influenced. In what manner is this accomplished and how is the cochlea affected by this displacement?

In the experiments on the stimulation of the N. facialis in the pigeon, the cochlear area was necessarily thoroughly exposed. Fine droplets of perilymph appeared on the surface through the small crevices in the bone occasioned by the dissection. These tiny holes were also intentionally produced by boring into the bony wall of the cochlea with a needle, and with the same result. When the N. facialis trunk was stimulated the small drops of perilymph were immediately sucked into the cochlea and would reappear as soon as the stimulation ceased. The contraction of the M. tensor tympani therefore tends to reduce the pressure within the perilymphatic space.

If one opens the cochlea from its cranial aspect and removes the membranous labyrinth, one may readily observe the columellar foot-plate. If the tendon of the M. tensor tympani is pulled on, one may see that the proximal anterior border of the columellar plate appears to be displaced. I have used the word 'appears' because the traction upon the tendon of the muscle in the manner described is, after all, a rough experiment but the displacement is quite probable from the anatomical relation of the parts themselves.

It does not appear possible to homologize this muscle with the intrinsic ear muscles of the mammals. A comparison with the M. retractor auriculæ is more readily accomplished. A functional comparison is however readily made. It seems certain that a muscle which attaches to the head of the columella; draws it backwards; displaces the stapedial plate out of the fenestra vestibuli; and reduces the pressure within the labyrinth—is a complete functional analogue of the mam-

malian *M. stapedius*. But this muscle is, at the same time, one which increases the tension of the drum membrane. Functions, which in mammals, are divided between two antagonistic muscles, are therefore accomplished in birds by a single muscle which not only renders the drum more tense but also decreases the intra-labyrinthine pressure. If both of these functions may be merged in the bird, the *M. tensor tympani* and *M. stapedius* of the mammals cannot properly be classed as muscles of opposition.

Breuer's conclusions on the function of the *M. tensor tympani* in birds are:

That it combines the action of the mammalian *M. tensor tympani* and *M. stapedius* in that it increases the drum tension and at the same time decreases intra-labyrinthine pressure; second, because the single muscle in birds replaces the double muscle in mammals, the two muscles in the mammal cannot well be regarded as opponents but rather as synergists; third, the *M. tensor tympani* possesses no muscle of opposition, and its contractions therefore have to do with preserving its tonus, with maintaining the pliability of the columella and in particular, the annular ligament attaching the columellar foot-plate to the margin of the fenestra vestibuli; fourth, to compensate for minor mechanical errors in the sound-transmitting apparatus (the nature of which he does not suggest).

Beyer ('07) describes the drum membrane in greater detail and particularly in the matter of its attachments. An annulus is not found in birds and the membrane is therefore attached to the adjacent borders of the surrounding bones; the pars basilaris of the sphenoid, the os occipitale laterale and basale, the squamosum, and the tympanic process of the quadratum. The last-named bone is movable, and may therefore influence the drum.

#### STATEMENT OF THE PROBLEM

Little work, apart from the notation in Bronn ('93), has been done on the tuba auditiva. Here the common tubal orifice is described as occupying a median position in the occipitosphenoidal suture. The right and left tubae fuse into a short common tube or duct which opens into the posterior part of the oral cavity.

It occurred to the writer ('14) that little work had been done on the mechanical factors involved in the middle-ear region of

birds, and attention was then called to the position of certain elastic ligaments which are practically constant in all birds examined. More detailed investigation has been undertaken to ascertain the functional relation of these elastic ligaments to the action of the *M. tensor tympani*, to the drum membrane, and to the columellar apparatus. Breuer has undoubtedly described the muscle correctly as one without an opponent, and the structure should be of interest to the physiologist in determining the possibility of a dual nerve supply; an excitor-inhibitor system as found in involuntary muscles or in the voluntary muscles with opponents as proposed in Sherrington's reciprocal innervation.

The scope of this paper, based almost entirely on Gallus, will be limited, first, to the description of the elastic elements of this region and to the mechanical features in the columellar system; second, to an interpretation of the mechanism of the bird's middle ear.

The material used consists of newborn and semiadult chickens, fixed in Bouin's fluid or in formalin, decalcified in hydrochloric acid-alcohol and sectioned in collodion or paraffin. The sections were stained in Weigert's resorcin-fuchsin or in orcein, and for the most part differentiated in acid alcohol. The collodion sections were all cut at  $50\mu$ , the paraffin sections at  $25\mu$ .

#### ELASTIC TISSUE OF THE EXTERNAL AUDITORY CANAL

The external auditory canal in the chicken leads obliquely downward and backward from its oval external opening which is protected by the upward projection of the lower bordering feathers. The orifice lies well in front of the short bony external canal and is situated in a plane at right angles to the position of the drum membrane. The canal has a distinct kink in it and particularly the ventral-median wall is bent upon itself so that the most external part is applied to the side of the head. The dorsal wall of the canal is longer than the ventral, because of the oblique position of the drum membrane, and is reinforced by a heavy fibrous plate which is attached to the edges of the bones surrounding the region, with the exception of the quadratum.

This fibrous plate, bent to conform to the outline of the canal, affords origin to the heavy *M. digastricus*. The lateral portion of the canal is freely movable, so that the bird may shear off communication from the surface to the drum at the point of the kinking. The skin of the canal is loosely applied, particularly over the fibrous plate which attaches the *M. digastricus*, where a bursa-like slit occurs which continues to the region of the drum. Traction upon the skin of the external canal does not affect the drum membrane, and it may be assumed that the contraction of the *M. digastricus* or the closing of the external auditory canal in no way influences the middle ear or its contents.

The drum membrane occupies the upper wall of the canal and looks downward and somewhat backward. It is well hidden from inspection by the kinking in the canal and the convexity of the fibrous plate. This is further accentuated by the presence of the erectile auditory pad — a semicircular worm-like elevation following the dorsal drum margin and located about 2 mm. lateral to it. The erectile pad is firmly attached at the upper and lower bony borders of the external canal, but its semicircular base is separated from the canal by the bursa-like slit. In section this pad (fig. 1) is a rugous structure, largely composed of connective-tissue elements and some venous spaces. It is limited at its base by stout elastic fibers which are attached to the periosteum near the drum margin, but are not continuous with the drum membrane (fig. 2). The structure was first described by Wurm ('85) as responsible for the deafness in *Tetroa urogallus* during the period of sexual excitement in that it plugs the external auditory canal through turgescence. This was substantiated by von Graaf ('85), who, however, questions the additional contributing factor suggested by Wurm in a pressure exerted by the processus angularis mandibulae against the external canal. The pad itself partakes of the age and sex differences displayed in the comb and wattles, and is larger in the adult than the young bird, and better developed in males than in females. The bursa-like slit separates the pad from the tendinous fibers of the *M. tensor tympani* which crosses its base at right angles near the drum margin.



Turgescence of the pad might readily displace it forward and outward and seal the external auditory canal, as Wurm has suggested, but the position of its elastic fibers would indicate that they are related to a pressure against the medial surface of the pad and might therefore afford support to the drum mem-

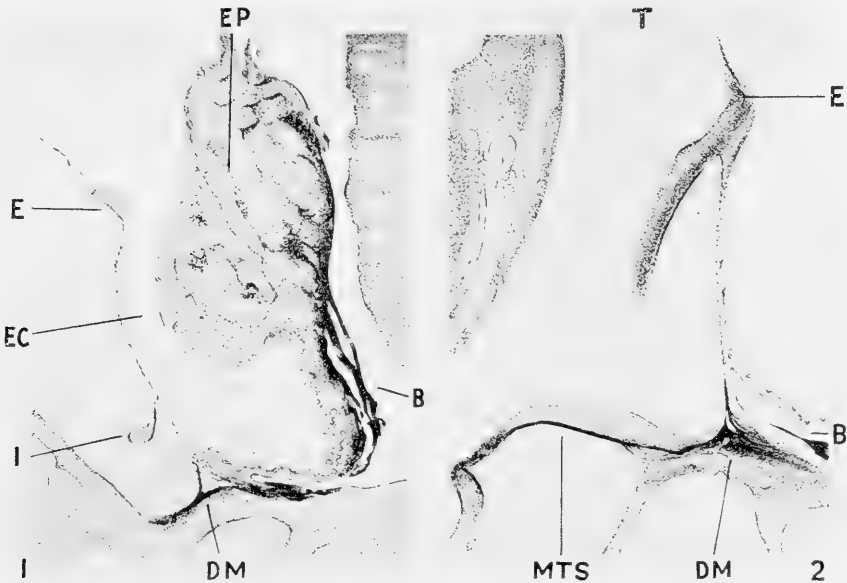


Fig. 1 Section through the dorsal limit of the drum membrane and external auditory canal of an adult chicken; orcein stain. *B*, bursa; *DM*, drum marginal elastic tissue; *E*, extrastapedial; *EC*, external auditory canal; *EP*, erectile pad; *I*, infrastapedial.

The stippled figures are drawn by Miss Gertrude Hance of St. Louis.

Fig. 2 Oblique section through the dorsal region of an adult chicken; resorcin-fuchsin stain. *B*, bursa; *DM*, drum marginal elastic tissue; *E*, extrastapedial; *MTS*, membrana tympani secundaria; *T*, tympanum.

brane or columella against a too-marked lateral excursion. The elastic fibers would tend to draw the pad inward and backward and assist in returning the blood into the jugular vein, or would tend to bring the pad back to the normal position when the drum membrane returns to its usual location. The glands of the external auditory canal probably secrete a defensive mate-

rial against the intrusion of vermin, and because of the shearing mechanism and the erectile pad, the bird undoubtedly possesses a more adequate protection against violence from without than does the mammal.

While the fibrous elements of the ventral wall of the external auditory canal swing around the prominence indicating the position of the tympanic process of the quadratum, to terminate in close relation to the drum attachment, this is not the case at the dorsal drum margin, which is separated from the fibrous plate by a bony lip which contributes to a rudimentary external osseous canal. This lip is perforated by a foramen which transmits the tendon of the *M. tensor tympani* from the external surface of the skull to the dorsal-inferior drum margin. The muscle has been correctly described by Killian, figured correctly by Breschet, and is clearly innervated through the *N. facialis* as the experiments of Breuer substantiate. The relation of the tendon of this muscle to the drum membrane and the function of the *M. tensor tympani* will be considered later. The pull of the muscle, judging from its attachments and structure, is probably limited and feeble.

The drum is attached to the margins of the bones surrounding the external canal, but in the case of the quadratum this does not appear to be the case, although Beyer has noted it explicitly. The attachment of the drum, at this region, is to a stout fibrous ligament which rides over the rounded surface of the tympanic process of the quadratum near its articulation with the squamosum, and at this portion of the drum circumference, a diverticulum of the middle ear appears to afford a sort of cushion which insulates the drum membrane from the small movements in the quadratum.

#### ELASTIC ELEMENTS OF THE DRUM MEMBRANE

The drum membrane has been well described by Breuer and by Beyer. It consists of a delicate oval membrane attached to the bones limiting the tympanic cavity, with the exception of the quadratum anteriorly. The movements of the tympanic

process of the quadratum, which is directed upward in front of the drum membrane to articulate with the squamosum, are mainly a fore-and-back hinge motion and very limited near the joint. It must be remembered that the quadratum is intermediate in position between the mandible and the squamosum, and because of its attachments through the zygomatic also participates in movement of the upper bill. However, the bone is limited in motion through attachment to the pterygoideum, and in birds with a freely movable upper bill, as in the parrot, the quadratosquamosal articulation is even more pronouncedly a hinge than in birds with little upper bill movement, as in the chicken. A stout ligament usually bridges the tympanic process of the quadratum at the drum margin. In any event when the quadratum is freed below, it may be violently wrenched out of its socket without any visible influence on drum tension or columellar position, even when the performance is observed under binocular magnifier. The drum attachments are therefore probably as stable as if an annulus were present, and the movement of the quadratum in reference to the tympanum may be quite disregarded, as far as the chicken is concerned.

The drum margin is thickened by intrinsic elastic fibers, and in the ventral attachment area, includes a rudimentary air sinus which was at first mistaken for a vein. It is possibly a rudiment of a siphonium canal which connects the air sinuses of the mandible, when they are present, with the cavity of the middle ear just inferior to the foramen pneumaticum for the quadratum and pterygoideum. The marginal sinus, indicated in the semischematic figure 4, in so far as the writer is aware, has not been described, and the result of its position in relation to insulating the drum margin from movement of the quadratum has already been mentioned.

The margin of the drum is thickened postero-inferiorly, where the tendinous fibers of the *M. tensor tympani* pass to their attachment to the extra- and infrastapedial cartilages. Additional elastic elements are related to the tendinous portion of this muscle, and the drum margin and *membrana tympani secundaria* appear to be more or less continuous at this point.

The drum, in addition, receives strengthening fibers from definite elastic ligaments which may well be named the drum-tubal ligaments; these will be discussed after the general structure of the columella has been considered, because they are definitely related to the columella, both in position and in function.

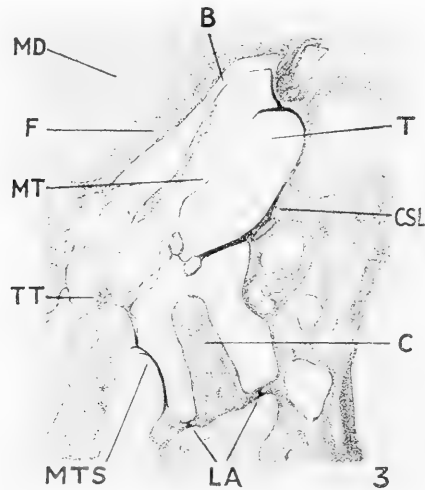


Fig. 3 Section through the length of the columellar recess of a twenty-day chick; resorcin-fuchsin stain. *C*, columella; *CSL*, columellar-squamosal ligament; *F*, fibrous tube of external canal; *LA*, ligamentum annulare; *MD*, musculus digastricus; *MT*, membrana tympani; *MTS*, membrana tympani secundaria; *T*, tympanum; *TT*, tendon of tensor tympani.

#### COLUMELLAR APPARATUS

The strut responsible for the prominence of the drum in the external auditory canal is the columellar apparatus, which demands a careful consideration and which may be divided into two segments, a medial bony columella proper and a lateral cartilaginous extracolumella.

The bony columella has been likened to the mammalian stapes with a long stalk attached to it. It begins medially at the margin of the fenestra vestibuli as a flattened bony plate, the columellar foot-plate, which rapidly decreases in size to form a

slender spicule of bone which is directed laterally and somewhat downward to terminate in the extracolumella. It is flattened from before backward as it approaches its lateral extremity. The columella occupies a recess formed by a diverticulum of the

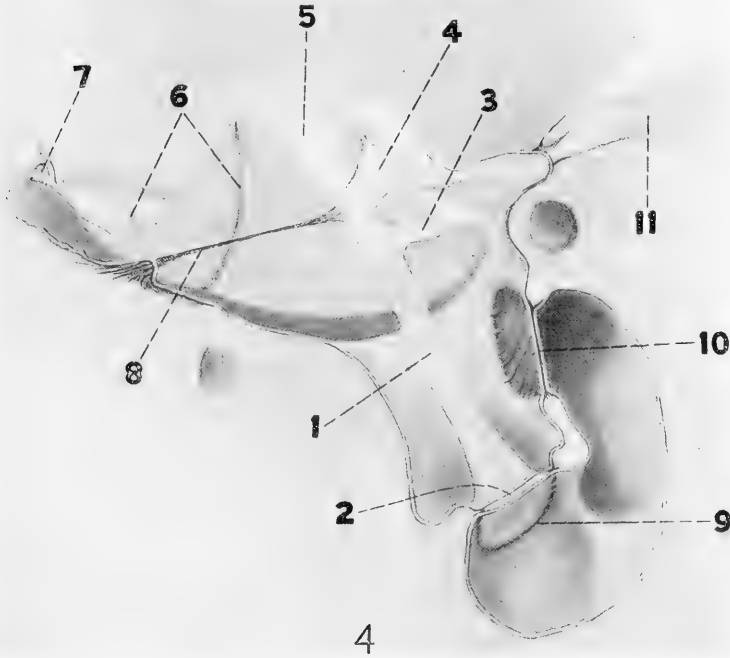


Fig. 4 Dissection of the middle ear of an adult chicken, somewhat schematized. 1, columella; 2, columellar foot-plate; 3, infrastapedial; 4, suprastapedial; 5, extrastapedial; 6, middle drum-tubal ligament; 7, drum-marginal air sinus; 8, columellar-squamosal ligament; 9, ligamentum annulare; 10, membrana tympani secundaria; 11, M. tensor tympani.

tympanic cavity under the carotid canal, ossifies early, and, in the young bird, is relative plump and better developed than the extracolumella (fig. 3).

The description of the extracolumella is more difficult because it consists of a cartilage tripod with the three processes set at right angles to each other. Figure 4, drawn from the actual dissection, gives the general form of the structure fairly well in

an adult bird. The three processes have received many names of which, perhaps, infrastapedial, suprastapedial, and extrastapedial are as good as any. We may refer to the common origin of these processes as the common cartilage stalk of the extracolumella. The infrastapedial (3) leaves the common cartilage stalk at its union with the columella and extends downward, at a right angle to the position of the columella, to terminate in a somewhat sharpened extremity close to the drum margin. The attachment of the columella to the extracolumella is somewhat flattened in the axis of the infrastapedial, and results in a movable member in this axis, which may be termed 'the columellar hinge.' The suprastapedial (4) is a spatulate process with its pointed extremity downward and forward, where it attaches to the common cartilage stalk, and its widened area correspondingly upward and backward where it attains the postero-superior drum margin. The flattened surface is at right angles to the columellar axis and the plane of its position is about the same as that of the infrastapedial. This process is not only the largest and most firmly attached portion of the extracolumella, but its base, attached to the drum margin, constitutes another hinge member which may be called the 'suprastapedial hinge.' The movement of this hinge is necessarily accompanied by drum displacement, and, in a general way, is outward-downward and inward-upward. The extrastapedial process (5) arises from the cartilage stalk, nearly in the axis of the bony columella, and lateral to the confluence of the infra- and suprastapedials. This process acts like the center pole of a tent and gives the drum membrane its curious inverted umbo to which attention has already been called. The extrastapedial receives most of the fibers of the M. tensor tympani, the drum course of which can readily be identified by inspection; sometimes the extrastapedial cartilage is particularly well developed out along the drum in the direction of the tendinous fibers. This process varies markedly in different ages and in different species of birds. Its greatest movement occurs at the union of the three cartilage processes and is a hinge, practically in the plane of the action of the M. tensor tympani. These hinge areas in

the columella are of some little importance in interpreting the mechanics of the columellar system, and will be referred to later.

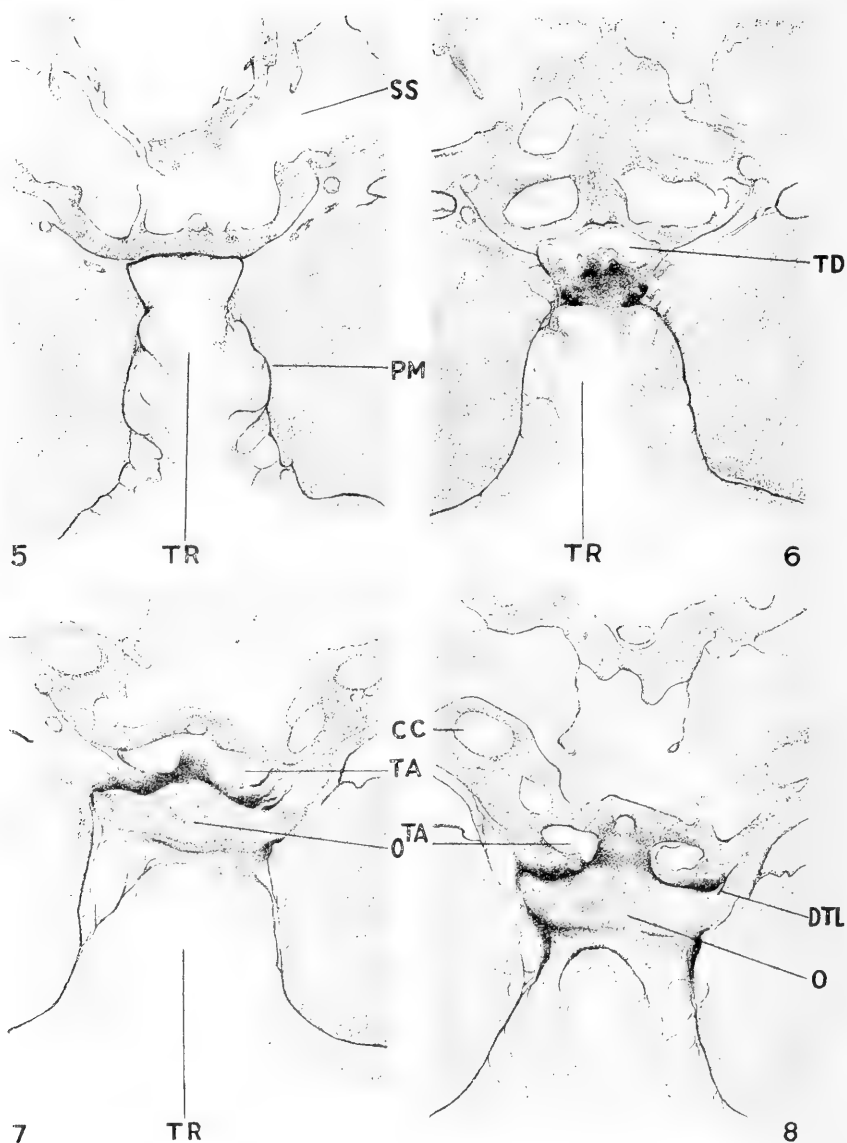
#### DRUM-TUBAL LIGAMENTS

Medial inspection of the drum membrane, particularly in a specimen stained in toto with orcein and differentiated in alcohol, reveals a series of elastic ligaments which were pictured by Breschet and rediscovered by Smith, although neither investigator apparently understood their nature or function. These ligaments may well be named the drum-tubal ligaments and described in drum and tubal segments. The superior and middle drum-tubal ligaments arise from a common strand of elastic tissue in the lateral wall of the tuba auditiva and in close relation to the drum margin. The superior ligament joins the drum margin and runs upward, contributing to the medial elastic wall of the drum marginal sinus. It may be traced to the quadratosquamosal joint or to the foramen pneumaticum for the quadratum.

The smaller segment, the middle drum-tubal ligament (fig. 4), is the pearly streak of Breschet, and is readily observed in most birds, and in all instances is distinctly fused to the drum tissue and not independent in the turkey, as Breschet described it. It terminates in the tip of the extrastapedial and forms a very obtuse angle with the line of the pull of the *M. tensor tympani* on this process. It seems to become less markedly developed as it passes across the drum surface.

The inferior drum-tubal ligament arises from the floor of the tuba auditiva, and while it contributes some fibers to the inferior drum margin, the major portion passes across the edge of the drum to terminate in the tip of the infrastapedial which, it will be recalled, does not quite reach the edge of the drum. This ligament is ordinarily better developed than the other two combined (not shown in figure 4).

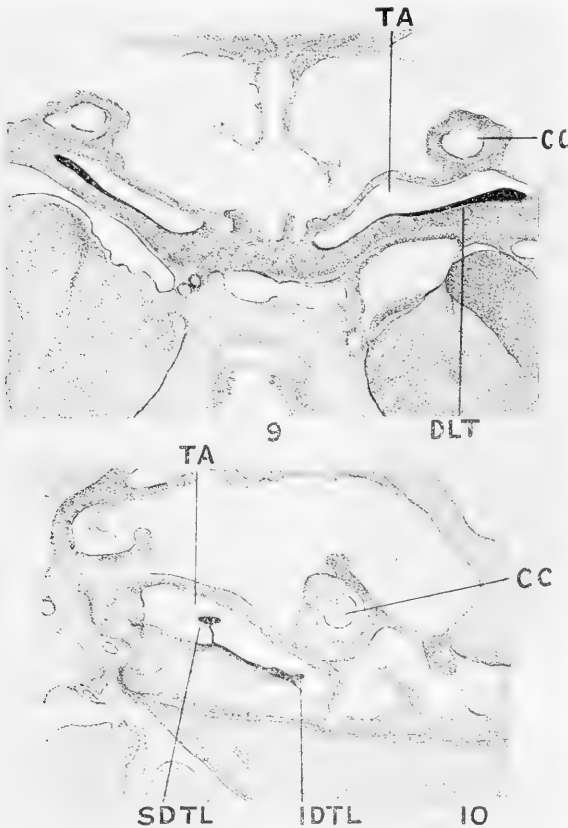
The tuba auditiva therefore presents two elastic strands near the drum margin, the inferior drum-tubal ligament and the fused superior and middle drum-tubal ligaments. These two join to form a common elastic bundle before the tuba has crossed



Figs. 5 to 10 CC, carotid canal; DTL, common drum-tubal ligament; IDTL, inferior drum-tubal ligament; O, projecting lip of the occipitale; PM, pharyngeal membrane; SDTL, fused superior and middle drum-tubal ligaments; SS, sphenoidal sinus; TD, common tubal duct; TA, tuba auditiva.



the plane of the carotid canal and lie in the lateral tubal wall. The tube becomes rapidly smaller as it proceeds medially, and when the tube fuses with its fellow to form the common duct already mentioned, the right and left elastic strands also fuse to



form the floor of the common opening in the occipitosphenoidal suture. Here the amount of elastic tissue is increased by fusion with the stout elastic pharyngeal membrane which is projected upward into the tubal recess between the palatal muscles. This may be followed upward from the recess in figures 5 to 10, inclusive. It may be interesting to note that in certain birds (goose,

duck) the tubal course of these ligaments is replaced largely with involuntary muscle which becomes continuous with the involuntary muscle at the common tubal orifice. Breschet pictures the ligaments, but his account of the influence of traction upon these ligaments or the indirect traction through the mus-

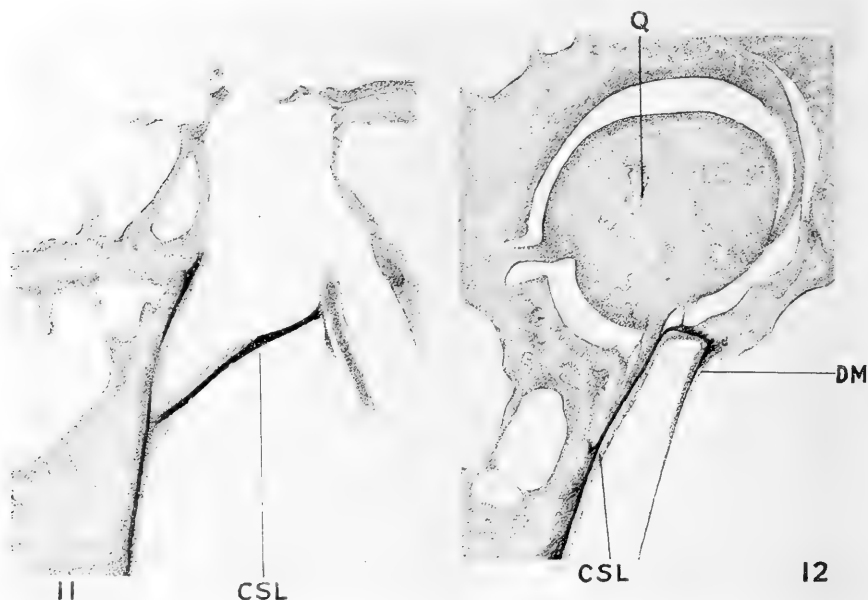


Fig. 11 Section through the length of the columellar-squamosal ligament of an adult chicken; orcein stain.

Fig. 12 Section through the quadrato-squamosal articulation of an adult chicken, showing the relation of the head of the quadrate (*Q*) to the fibers of the columellar-squamosal ligament; resorcin stain.

cles of the mandible attached to the tubal wall must be denied. The bird does not possess the cartilaginous tuba auditiva of the mammal. The function of the drum-tubal ligaments is clearly one of limiting the amount of excursion in the extra-stapedial, in particular the infrastapedial process, and will be considered later.

While birds undoubtedly close the tubal recess during the act of swallowing, a direct relation of what corresponds to the raising of the soft palate and its effect on the cartilaginous tuba auditiva in the mammals cannot obtain. This region demands further study. It is interesting to note the relatively small tubae auditivae of the bird, when compared with the relatively large air-sinus system, and in figure 6 the confluence of the air-sinus system may be noted in a twenty-day chick, which would tend to show that air sinuses are a result of bone absorption rather than a causal factor.

#### COLUMELLAR-SQUAMOSAL LIGAMENT

This ligament was also pictured by Breschet and described by Platner ('39), hence commonly known as Platner's ligament. It is a well-developed elastic band somewhat conical in form. Its apex is attached to the suprastapedial and the common cartilage stalk. It is directed forward, at right angles to the columella, to become attached by a broad conical base at the region of the quadratosquamosal articulation (fig. 11). While it is usually described as attached to the quadratum, its strong attachment appears to be to the squamosum, and it seems to ride over the quadratosquamosal joint to become continuous with the elastic tissue of the drum margin and with the elastic ligaments of this articulation. It is sometimes well separated from its bony attachment by an air-sinus opening. In very young birds the ligament appears to be placed almost at right angles to the position of the middle drum-tubal ligament, and while the ligament is in part set in opposition to the pull of the *M. tensor tympani*, its chief function seems to be to afford support to the extracolumella, so it may be considered a third member of extracolumellar stability; the other two being formed by the supra- and infrastapedials. All three of these members are attached just medial to the drum margin and the three form a somewhat elastic support to the extrastapedial which projects beyond the plane of the drum margin and is therefore responsible for the convexity of that membrane outward. This also accounts for the fact that drum convexity is maintained when the bony

columella has been severed. While the columellar-squamosal ligament resists the pull of the M. tensor tympani, and therefore is a passive agent in preventing outward displacement of the drum, it also affords certain resistance to medial displacement in the same manner that three toothpicks may be placed over the opening of an ordinary drinking glass. This ligament is indicated in figure 4 and is shown in figure 3.

The columellar processes have, therefore, definite elastic ligaments associated with them. The infrastapedial attaches the inferior drum-tubal ligament; the suprastapedial, the columellar-squamosal ligament, and the extrastapedial, the middle drum-tubal ligament. All of these ligaments are seen in a gross dissection and the forces which bring about an increase in tension may readily be demonstrated.

Occasionally additional ligaments occur. Sometimes a delicate elastic strand extends directly across the tympanum, from the quadratosquamosal articulation to the drum proper, and this may be regarded as an aberrant portion of the columellar-squamosal ligament; more often the fibers of the inferior drum-tubal ligament are projected up the length of the infrastapedial. In spite of the close relation of the columellar-squamosa ligament to the quadratosquamosal articulation, there does not appear to be a transfer of motion from the quadratum to the columella. With the exception of the middle drum-tubal ligament, which is wanting or poorly developed in the pigeon, the ligaments as described seem to hold for all birds examined—chicken, duck, goose, turkey, and hawk.

#### ELASTIC LIGAMENTS OF THE FENESTRA VESTIBULI AND FENESTRA COCHLEAE

The perilymphatic space of the inner ear is separated from the cavum tympani at the fenestra vestibuli by the columellar footplate which is held in place by an annular ligament, much in the same manner as the basis of the stapes is attached by the ligamentum annulare in the mammal. The margin of the footplate practically fits the fenestra vestibuli, and the interval is bridged by short stout elastic fibers, which permit a limited

inward and outward movement. It is perfectly obvious that the motions in the columellar foot-plate must be accompanied by corresponding mass displacement of the perilymph and that the plus or minus pressure developed would be in direct proportion to the elasticity of the tissues which limit that space. Breuer has called attention to the possible function of the M. tensor tympani in maintaining a pliability of the annular ligament, but from the elastic nature of its structure it may be safely assumed that exercise in no way contributes to this property. Whether the foot-plate motion is a direct tilting, after the manner of the basis of the stapes, or whether it is a direct in-and-out displacement is not germane at this point. The position of the elastic fibers are indicated in figures 3 and 4.

The fenestra cochleae in birds is relatively larger in proportion to the size of the perilymphatic space and of the fenestra vestibuli than in mammals. It is placed on the posterior aspect of the columellar recess in the chicken, and extends almost from the margin of the fenestra vestibuli to the drum attachment in baby chicks, although in older birds the growth of bone separates it more and more from the drum membrane. The membrane which closes in this opening, the membrana tympani secundaria, is composed of long, interlacing elastic fibers, which attach for the most part directly to the lip of the fenestra. This membrane is undoubtedly an adaptation for compensative displacements in the perilymphatic fluid, due to the direct application of a movable skeletal element to this space. This same mechanical requirement would hold for the operculum in the amphibians; the columellar foot-plate in the birds, and the basis of the stapes in the mammals, and would imply that somewhat similar functional requirements might obtain in these widely separated forms. It is interesting to note that the membrana tympani secundaria in the amphibians seems to be composed of elastic tissue, although the membrane occupies a dehiscence at the base of the otic capsule (as H. S. Harrison ('03) has shown) and is applied to a lymph sinus in this situation. The membrana tympani secundaria in the amphibians therefore has no direct relation to the tympanic cavity. Similarly, the

operculum is fixed in place, partly by a hinge in the cartilage and also by a stout elastic ligament which appears to have a functional relation to the *M. opercularis* as determined by Ahearn in an unpublished thesis on the anuran ear.

#### MECHANICS OF THE COLUMELLA AND EXTRACOLUMELLA

The results of the contraction of the *M. tensor tympani* are apparently in accord with Breuer's statement—downward, backward, outward displacement of the extrastapedial and an increase in tension of the anterior-superior drum quadrants. The changes in the drum membrane can naturally only come about through displacement of the extracolumella. The mechanics of this displacement and its effect upon the columella demand a more detailed description. The writer does not feel that drum tension itself has any very important relation to the problem, but rather that it is a result of adjustment of drum position.

Close inspection of the columellar apparatus *in situ* reveals four distinct kinds of movement. The first of these is a more or less typical plunger motion of the columellar foot-plate in and out of the fenestra vestibuli. The other three are hinge-like in character and are dependent either on the displacement of the extracolumella as a whole or upon bendings which take place in the extracolumella itself. These three movements will be described under the terms columellar hinge, suprastapedial hinge, and extrastapedial hinge. It must of course be remembered that the hinges in the cartilage are much of the same character as the operculum hinge in amphibians—merely weakened areas which permit a bending and not true joints.

The movement of the columellar foot-plate in the fenestra vestibuli is more or less an in-and-out motion probably combined with a slight tilting as found in the mammalian stapes. Unlike the condition in the mammal, however, where the force is directly applied to the bony stapes, the movement is imparted to the columellar foot-plate through the bony columellar lever by a tilting of the elastic extracolumella. The result of this is a final direct push and pull of the relatively long bony columella upon its foot-plate, or a resultant limitation of the tilting which

is so characteristic in the mammal. The amount of medial and lateral excursion of the columellar foot-plate is limited by a number of factors: 1) The shortness of the stout elastic fibers of the ligamentum annulare which is quite evenly developed about the circumference of the foot-plate, with the possible exception of the extension of fibers toward the drum margin; 2) by a compensating tension of the membrana tympani secundaria which allows a mass displacement of inner-ear fluid as an adjustment to movements of the foot-plate; 3) by limitations in a medial and lateral excursion because of the position of the columella in its attachment to the extracolumella and the restrictions in the tilting of the latter. Breuer's experiments on the living pigeon substantiate, what appears obvious from dissections, that contractions of the *M. tensor tympani* result in displacement of the extracolumella and a slight lateral excursion of the columellar foot plate. The result of this movement will be discussed later.

The second point of motion is in what may be termed the columellar hinge, located at the point of union of the lateral tip of the columella with the extracolumella, immediately medial to the point where the infrastapedial process is given off. Here the form of the columella changes from a rounded to a flattened spicule—the flattening being in the axis of the infrastapedial process. This hinge represents the spot where the tilting action of the extracolumella is translated to a plunger action of the columella proper and is accompanied by certain resisting forces; the twisting of the infrastapedial and the tension on the elastic columellar-squamosal ligament. It will be recalled that the infrastapedial has attached at its tip, or inferior end, not only the tendinous fibers of the *M. tensor tympani*, but the inferior drum-tubal ligament as well. The twist in the infrastapedial is one of the factors which tends to return the columella to a position of rest on muscular relaxation, as noted by Breschet.

The third point of motion occurs when the extracolumella, as a whole, swings upon a hinge formed by the attachment of the suprastapedial to the drum margin. This hinge is reinforced by the tip of the infrastapedial and perhaps also by a long drum process of the extrastapedial. The movement consists in an out-

ward, backward, and downward tilting of the entire extracolumella and is limited, as far as the downward movement is concerned, by the infrastapedial and the direction of the force exerted by the M. tensor tympani. The outward and downward motion is controlled through the attachment of the columellar squamosal ligament, plus the indirect pull of the M. tensor tympani on the extrastapedial, and the thickness of the adjacent drum quadrant.

The fourth hinge member is formed at the point of attachment of the extrastapedial to the common cartilage stalk, just lateral to the confluence of the supra- and infrastapedial processes. This process is directed laterally and is responsible for the drum prominence, because it acts like a strut which is supported by the supra- and infrastapedial and the columellar squamosal ligament. The cartilage bends downward and backward as it touches the drum, and may continue in the direction of the attached tendinous fibers of the M. tensor tympani as far as the drum margin. The pull of the muscle is directed mainly on the extrastapedial, and when stability is attained in the plane of the three members—supra- and infrastapedial and columellar squamosal ligament—further displacement may occur by a tilting of the extrastapedial at its basal attachment. This motion is limited, in part, by the form of the extrastapedial and by the middle drum-tubal ligament. The movement is pronounced in the chick, but poorly developed in the turkey, where the extrastapedial is relatively heavy and the middle drum-tubal ligament well developed.

#### FUNCTIONAL RELATION OF COLUMELLAR APPARATUS AND M. TENSOR TYMPANI TO THE INNER EAR

The entire elastic-ligament system is directly related to the movements in these four areas, and must therefore be set in opposition to the forces which bring about these movements. The mechanism is one which allows for marked alterations in drum position, and therefore in the sound-transmitting apparatus, without materially influencing the pressure within the perilymphatic space. It would appear these bending areas are



compensatory to drum movements and are probably not primarily associated with acuteness in hearing. The variable factor which allows the single voluntary *M. tensor tympani* to remain in tonus against the constant pull of elastic ligaments is logically, therefore, a function associated with the middle ear. This factor is necessarily a variable one and must involve a change in the topography of the middle-ear region which requires adjustment.

This attempt to look upon the *M. tensor tympani* and the columellar system as adjustment factors to a variable middle-ear topography calls for a short account of the adjustment necessarily involving inner-ear mechanism. It is not my purpose to consider the inner-ear function except as it pertains to middle-ear mechanics.

Keith dismisses the mechanical factors in the middle-ear region of birds in a few words. His work deals mainly with the problem of the inner-ear adjustments to columellar movements, and his evidence was obtained largely from the sparrow.

In the case of the bird, a single bone—the columella—connects the drum with the oval window. While the outer end, of the columella is fixed to the drum, its inner end extends into a foot-plate which is fixed into the margin of the oval window by a ligamentous membrane. The lower and hinder borders of the foot-plate are more tightly fixed in the window than the upper and anterior margin, not unlike the manner in which the stapes is attached in the fenestra ovalis of the mammalian ear. The movements of the columella are more like those of a lever than of a piston; it is hinged to the lower margin of the oval window. Internal to the foot-plate is the cavity of the vestibule, filled with fluid. The horizontal partition is drawn across the floor of the vestibule, stretching from the lower margin of the oval window which is occupied by the foot-plate to the opposite or inner wall of the vestibule (p. 222).

It will be noted that the middle passage (*scala media*) of the cochlea is separated from the cavity of the vestibule by a thick folded membrane containing many blood vessels—the *tegmentum vasculosum*. It represents a combination of the Reissner's membrane and the vascular body (*stria vascularis*) of the mammalian ear.

The horizontal partition just described forms the floor of the vestibule and the roof of the lower or tympanic passage of the cochlea—at least the terminal part of that passage. The round window lies immediately below the oval window. It is closed by a strong but loose membrane which is placed between the lower end of the tympanic

passage and the cavity of the middle ear or tympanum. Now, in considering the mechanism of the bird's ear, we may omit from our calculations the movements of the tegmentum vasculosum; it is a slack membrane and may be regarded for our present purposes as forming part of the fluid which fills the vestibule. When, then, the outer end of the columella is set into vibration by the drum, its foot-plate will carry the impulses of the drum to the fluid filling the vestibule. As in the mammalian ear, we may distinguish four phases in each vibrational cycle. In phase I the foot-plate, starting from its point of rest, moves or rotates inwards, displacing a minute quantity of the vestibular fluid. The vestibule has firm walls everywhere except that part of its floor formed by the basilar membrane. The membrane yields, displacing the fluid contents of the lower passage and forcing out the round membrane. In phase II, the foot-plate returns to its starting point, and the basilar membrane and organ of Corti rise to their equatorial level. In phase III, the outward excursion of the foot-plate continues and the basilar membrane rises so as to become convex upwards; the round membrane is drawn in. In phase IV all these parts return to rest.

Both Wrightson ('18) and Keith ('18) in their discussion have attempted to make the anatomy and physiology conform to the theory rather than analyze the theory on the basis of structure and function. It would appear from Wrightson's account that the pressure of the fluid in the perilymphatic and endolymphatic spaces is atmospheric and that the opposed action of the M. tensor tympani and M. stapedius preserves this condition. It has already been pointed out that the most recent investigations declare for a synergistic action (Kato, '13) of the muscles named. Keith, in any event, makes no suggestion as to the opponent for the single M. tensor tympani in birds, nor does he even mention the unusual mammals in which only one muscle is found. It must be conceded that the intralabyrinthine pressure is probably controlled by secretion and by blood pressure, and that the control exerted by the muscles of the middle ear is merely transient and secondary.

This again brings up several points which have been disregarded by Keith: The bony labyrinth is not a closed container, but communicates freely to the outside through the ductus endolymphaticus and through blood-vessels, with which the region is richly supplied, with the general venous system. Many bird forms show no direct relation of the membrana tympani secundaria to the tympanic cavity, but to the vena jugularis.

While Keith notes the dehiscence in the otic capsule in the amphibians, as established by H. S. Harrison, he does not emphasize the fact that the *membrana tympani secundaria* in this form is applied to the base of the skull, outside of the territory of the *tuba auditiva* or that the middle-ear region of the anurans is distinctly respiratory in function, dilating with the injection of air into the lungs. While Keith suggests that we regard the *tegmen* *vasculosum* in birds and the *stria vascularis* in mammals as merely contributing to the fluid contents of the space, the writer feels they afford a most delicate adjustment to slight variations in pressure, owing to the fact the pressure within the labyrinth and the venous pressure is practically equal. Certainly, this regulation is more delicate than the bending of the *membrana tympani secundaria*, even granting this force to be less than the friction head against a mass displacement through the *helicotrema*.

It has been mentioned that a plane of stability is formed by the *suprastapedial* and *infrastapedial* processes and the *columellar-squamosal* ligament which attach on a line medial to the drum margin. This plane is responsible, in part, for the support to the *columella proper*. Breuer's experiments on the cochlea have demonstrated that when the cochlear wall is perforated, the perilymph tends to ooze out in the form of surface droplets and these droplets disappear on a stimulation of the *M. tensor tympani* through the *N. facialis*. The interpretation, however, that the resulting contraction of the muscle is designed to reduce intralabyrinthine pressure is, however, open to objection. The perilymphatic fluid undoubtedly has a pressure, as has been stated, which is about that of the capillary blood-vessels of this region, and the moment a hole is made in the bony canal, the fluid will naturally ooze out in the form of droplets, due also in part to the tendency of the *columellar apparatus* to be displaced inward on loss of tone in the *M. tensor tympani*. In fixed specimens the *membrana tympani secundaria* normally appears to bulge slightly into the *cavum tympani*, and is undoubtedly an artifact. The function of the muscle can be more properly associated with a push factor which tends to

press the columella into the fenestra vestibuli and thereby to beget increased perilymphatic pressure.

#### COMPARISON OF THE EAR REGION IN BIRDS AND MAMMALS

The region of the external and middle ear in birds differs in certain particulars from the corresponding regions in mammals. The external auditory canal in the bird may be closed to afford a direct protection against violence and pressure from without, whereas in mammals this is not possible. The drum membrane in birds, as indicated in the dead or anesthetized bird, appears to be displaced medially on relaxation of the *M. tensor tympani*, while in mammals relaxation of the muscle of the same name is accompanied by lateral displacement of the drum, carrying with it the malleus, probably on a rotation of the incus, the nature of which has not been clearly defined. The tympanic cavity and the large air-sinus system associated with it and the tuba auditiva, afford a relatively greater surface for air absorption in the bird than in the mammal. The two middle-ear cavities stand in open communication in the bird, while in the mammal they are distinct. The tubae auditivae in birds open directly, by a small common canal, into the posterior oral cavity, while in mammals the separate orifices lie above the level of the soft palate in relation to the posterior nasopharynx. In mammals the opening and closing of the tubal orifices is controlled by voluntary muscles associated with the muscles of the soft palate, while the shearing off of the common tubal recess is probably brought about by approximation of the bordering palatal folds. The *M. tensor tympani* and its closely associated *M. tensor veli palati* of the mammal are wanting in the bird, although the position of these muscles is occupied by the drum-tubal and columellar-squamosal ligaments. The *M. tensor tympani* of the bird corresponds to the *M. stapedius*, both in action and in nerve supply, except that the *M. stapedius* acts directly on the stapes, while the *M. tensor tympani* acts indirectly on the columella through a tilting of the extracolumella. Finally, in spite of the quadratosquamosal articulation and its close relation to the ventral drum margin and the tympanic cavity, the bird's middle

ear is probably as stable in the matter of fixed boundaries as is that of the mammals. Both groups have an arrangement in the sound-transmitting apparatus, which decreases the amount of drum excursion in the propagation of this motion to the perilymphatic space. The two joints in the mammalian ossicular chain are therefore replaced by a series of intrinsic columellar movements which have been spoken of as hinges. The lever action in the ear bones of the mammal finds a functional analogue in the columellar system in birds, and in neither instance is this movement probably associated with an attempt to transform the character of the sound wave. Recent evidence seems to point to the sound wave transmission as a molecular quantity.

While there can be little doubt that some changes in acuteness in hearing result from the contractions of the *M. tensor tympani* and *M. stapedius*, it is hardly fair to assume at this time that this is their prime function. A similar result is obtained in the eyes. Narrowing the palpebral fissure is accompanied by contraction of the pupil and therefore increase in sharpness of vision. This is, however, the normal sleep reflex and can scarcely be classed as a function of the lids.

If the bird and the mammal both enjoy a middle-ear region of relatively stable nature, there is at least one major factor which calls for muscular adjustment, and that is the variable topography of the drum in reference to the inner ear. It might therefore be well to review briefly some of the theories of the muscle function in the mammal. Wales ('09) has suggested the possibility of a plus pressure phase in the air content of the middle ear, which he holds is due to a forcible injection, caused by the method of tubal closure. This result is, however, the same as Mangold's ('13) observation in voluntary relaxation of the *M. tensor tympani*, apparently not accompanied by palatal motion, although, as has been suggested, it might be that the *M. tensor veli palati* might operate independently from the *M. levator palati* in these cases. In any event, the drum membrane undergoes a lateral displacement on relaxation of the *M. tensor tympani*, which has not been satisfactorily explained. The *M. stapedius*, according to Wales, would prevent a pressure of the

ossicular chain on the perilymph during the minus pressure phase (air absorption) in that it would hold the weight of the displacing incus from the stapes by transferring some of its thrust to the M. stapedius. The writer feels that, while Wales may have speculated on the changes in the pressure of the middle ear, the deductions in his article are both clever and suggestive.

The apparent double displacement factor in the mammalian middle ear makes difficult an actual observation of what takes place, under relatively normal conditions, although the cat seems to afford a material in which this may be done. The displacement of the drum membrane in the bird does not offer this objection, because the active muscular contraction operates in only one direction. The assumed plus pressure phase in the middle-ear region may therefore be constructed by creating a minus pressure in the external auditory canal, while the minus pressure phase may be imitated by increasing the pressure in the external canal. In either instance the middle ear may be thoroughly exposed without disturbing the drum or columellar apparatus, although the push-and-pull element on the columellar foot-plate and the membrana tympani secundaria is naturally diminished. This factor of error may be entirely eliminated by a simple change in technique in constructing a transparent roof for the tympanum and creating the pressure directly through the tuba auditiva. However, the conditions are fairly well reproduced in a gross dissection and are easily studied.

#### EVIDENCES OF ADJUSTMENTS TO DRUM MEMBRANE DISPLACEMENTS

By placing a carefully dissected specimen of a bird's middle-ear under binocular magnifier and gently aspirating the air from the external auditory canal, the following changes may be observed: The lateral displacement of the drum membrane is accompanied by a tilting outward and downward of the extracolumella on the suprastapedial hinge, supported in part by the infrastapedial, which is held from backward displacement by the tension on the

inferior drum-tubal ligament; the tilting action of the extracolumella is transformed into a plunger action of the columella at the columellar hinge, accompanied by a twisting of the infrastapedial on its long axis; the columellar-squamosal ligament becomes tense; the columellar foot-plate moves laterally at the fenestra vestibuli and the membrana tympani secundaria becomes concave to allow for displacement of the perilymph. If the aspiration be increased, the supra- and infrastapedials and columellar-squamosal ligament apparently create a stability in the extracolumella, so that further lateral displacement of the entire apparatus is eliminated, and the extrastapedial is drawn downward and backward in the direction of the tendinous fibers of the M. tensor tympani, until the middle drum-tubal ligament is under marked tension. At this time the extrastapedial is supported by the erectile auditory pad and the thin portion of the drum membrane applied to the anterior wall of the external auditory canal. This entire result implies that the amount of movement in the columellar foot-plate does not correspond to the amount of movement in the lateral displacement of the drum membrane, partly because of the hinge system in the columellar apparatus and partly because the extracolumella lies obliquely behind the midarea of the drum. Release of suction returns the parts to their former position.

Reversing the conditions by increasing the pressure in the external auditory canal, the drum moves medially, carrying with it the entire extracolumella on the suprastapedial hinge; the columella is displaced medially at the fenestra vestibuli and the membrana tympani secundaria becomes correspondingly convex. The medial displacement of the extracolumella is, however, limited by the close relation of the suprastapedial to the medial bony wall of the tympanum, and by the position of the intrastapedial and the columellar-squamosal ligament, so that further medial displacement of the drum occurs at the extrastapedial hinge. The extrastapedial buckles forward and inward, so that it may come to lie parallel to the columellar-squamosal ligament and, in the living bird, this is probably not so marked unless the M. tensor tympani relaxes to the utmost.

The delicate part of the drum is then applied to the medial wall of the tympanum. In other words, after a limited motion of the drum medially and laterally, all further movements are compensated for in the extracolumella itself, and therefore displacement of the drum does not give rise to corresponding displacement at the columellar foot-plate. It may be that the lever action ascribed to the three ear ossicles has the same purpose, rather than to function as transformers, because, in the bird, a similar arrangement is present and because, in the bird, the course from the tip of the extastapedial to the columellar foot-plate is practically a straight line.

The displacement which comes about through a minus pressure in the external auditory canal is practically equivalent to the action of the *M. tensor tympani*, while the plus pressure corresponds with its exaggerated relaxation. Therefore, the muscle might function to combat plus pressures in the external auditory canal, while the elastic-ligament system would passively counteract a plus pressure in the middle ear. It must be admitted, because of the relatively small tubal orifice and the large air content of the tympanum and its related sinuses, that birds are subject to as great, if not greater, displacements in the drum membrane as a result of barometric variations and altitudinal fluctuations. Relative increases in pressure against the drum would be shunted from the extracolumella through the contraction of the *M. tensor tympani*, while the reverse would be taken care of by the elastic ligaments. Therefore, because the drum membrane in birds normally tends to displace medially and press the columellar system against the perilymphatic space, a single muscle may operate. While in the mammal, according to Wales and Mangold, there is normally a tendency for the drum to be displaced outward which is controlled by the *M. tensor tympani*, while a medial displacement of the drum, carrying with it the ossicular chain, would press the stapes into the fenestra vestibuli and would be counteracted by the *M. stapediae*. It would appear, therefore, that the bird affords an excellent material for proving or disproving the importance of the columellar movements, the contraction of the *M. tensor*



tympani, and the displacement of the elastic ligaments against a variable topography in the middle-ear, which is due to drum displacements as a result of fluctuations in air pressure. This part of the problem clearly falls in the province of the physiologist.

#### CONCLUSIONS

The minor mechanical errors suggested by Breuer may prove to be the major factors calling for an adjustment, and in any event, the bird's middle-ear, with its relatively stable surroundings, its simple columellar apparatus, its single muscle, its elastic ligaments, and the possibility of shearing off its external auditory canal, is remarkably well adapted to varying conditions of air pressure. It would appear that the M. tensor tympani primarily compensates for variable drum positions in the columellar apparatus, rather than adapting the sound-transmitting mechanism to greater acuteness in hearing. The resulting drum tension would, therefore, be an incident to the contraction of the muscle, and in comparing conditions in bird and mammal, the greater the drum tension in the former, the more convex the drum; the greater the drum tension in the latter, the more concave the drum.

The prime function of the entire mechanism in birds would be represented by a function of the middle-ear, and compensatory to drum displacement, due to air absorption, on the one hand, and to fluctuations in barometric pressures on the other. The bendings in the columellar apparatus, while they are quite similar to the behavior of the ossicles in the mammal, probably do not have any effect upon the character of the sound wave. The sound wave, impinging upon the drum, would be focused, as it were, upon the stapedial or columellar foot-plate and beget vibrations of a molecular rather than of a molar character in the perilymph. The tension of the drum, like the shifting of the columellar foot-plate, is probably only a result of the variable topography of the drum in its relation to the inner ear and have little to do with acuteness in hearing.

Resumen por el autor, James Rollin Slonaker,  
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El desarrollo del ojo y sus partes accesorias en el gorrión (*Passer domesticus*).

En el presente trabajo ha intentado el autor dar una descripción condensada de la secuencia y modo de desarrollarse las diversas estructuras del ojo del gorrión, y relacionar estos hallazgos, tanto como sea posible, con el desarrollo de las estructuras estos hallazgos, tanto como sea posible, con el desarrollo de las estructuras correspondientes de otros animales, con referencia al orden de su aparición y también a la época en que se presentan primeramente. El trabajo está dividido en las siguientes secciones: el globo ocular; la córnea, iris y cámara acuosa; el cristalino; el cuerpo vítreo; las capas esclerótica y coroides; el peine (pecten); la retina; la fovea; los músculos del ojo; los párpados y las glándulas lacrimales. Teniendo en cuenta la diferencia del periodo de incubación, estas estructuras se desarrollan durante la misma época relativa en el gorrión y en el pollo. El trabajo va ilustrado con diez figuras en el texto y ciento cinco figuras en láminas.

Translation by José F. Nonidez  
Cornell Medical College, New York.

THE DEVELOPMENT OF THE EYE AND ITS ACCES-  
SORY PARTS IN THE ENGLISH SPARROW  
(PASSER DOMESTICUS)

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TEN TEXT-FIGURES AND SEVENTEEN PLATES (FIGURES 1 TO 105)

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INTRODUCTION

The published descriptions of the development of the eye of the bird have been largely confined to the domestic fowl, *Gallus domesticus*, which has an incubation period of twenty-one days. The incubation period of the English sparrow is about thirteen days—approximately that of a large number of our wild species.

The purpose of this paper is to describe the development of the eye and its accessory parts of the sparrow and to correlate the facts with the known conditions in the chick and other animals. In this manner it is hoped to ascertain if there be any difference in development of a domestic and a wild species. Domestication has caused changes of habits, and it may likewise

also produce modifications in structure. For example, it may be said that the eye of birds is characterized by the presence of a fovea for clear and distinct vision. The hen, so far as I know, is the only bird which does not possess a fovea, while the nearest related wild forms examined do (Slonaker, '97). Could the absence of a fovea in the hen have been brought about by domestication? If so, other structures may likewise have been modified.

The eye of the adult sparrow has been previously described (Slonaker, '18). This paper will deal mainly with macroscopical descriptions of the developing structures until they have reached the adult condition.

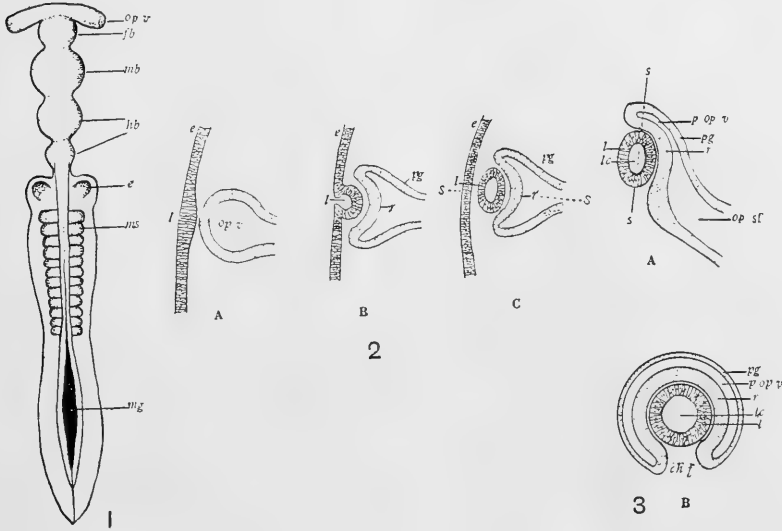
The material was secured from various nests. The eggs were thus in various stages of incubation. Eggs which were obviously fresh were placed in an incubator. In this way the size of the embryos at different ages was ascertained. The material which was collected in various stages of incubation was readily classified by comparison with this data.

The earliest stages of development of the sparrow were not secured. In order to show the early development of the eye of the bird, I have used the chick for the stages I failed to secure in the sparrow. This, I think, is permissible, since the eyes of all birds follow the same order of development.

All embryos were hardened in Perenyi's fluid and imbedded in celloidin. Serial sections were made and stained in haematoxylin and eosin. Perenyi's fluid preserved the retina in the most perfect condition of any hardening fluids tried, and at the same time it decalcified the bone wherever present. The sections of the embryos were made in a horizontal plane parallel to the lines *S-S* as shown in plate 1. In some of the older embryos the eyes were sectioned in both horizontal and vertical planes parallel to the axis of vision. Camera-lucida drawings were made wherever possible. All other drawings were made to scale.

## EYEBALL IN GENERAL

The development of the eyes in all vertebrates follow the same general plan. They first appear as lateral projections from the forebrain, known as the primary optic vesicles (text-fig. *opv*).



Text fig. 1 Dorsal view of the embryo chick at the age of about thirty hours. (See fig. 43.) *e*, auditory pit; *fb*, fore-brain; *hb*, hind-brain; *mb*, mid-brain; *mg*, medullary groove; *ms*, mesoblastic somites; *opv*, optic vesicle.

Text fig. 2 Diagrams showing the development of the lens and the optic cup. (See figs. 44 and 45.) *e*, ectoderm; *l*, lens; *opv*, optic vesicle; *pg*, posterior (pigment), and *r*, anterior (retinal) walls of the primary optic vesicle.

Text fig. 3 A, diagram of a vertical section of the eye of the chick at about forty-four hours' incubation in the plane *s-s* of figures 2, C, and 5. B, diagram of a section made vertical to A along the line *s-s*. (See fig. 45.) *chf*, chorioid fissure; *l*, lens; *lc*, lens vesicle; *opst*, optic stalk; *pg*, posterior (pigment) wall of the primary optic vesicle; *popv*, optic vesicle; *r*, anterior (retinal) wall of optic vesicle.

These vesicles continue their growth laterally until they come in contact with the ectoderm (text-fig. 2, A), the cells of which at this point become elongated (text-fig. 2, A *l*). This thickening is the beginning of the lens. Figure 43 is a photograph of a cross-section of a chick embryo of about forty hours' incubation. It shows the two optic vesicles (*Opv*) projecting laterally and

downward from the brain cavity. The right vesicle shows a noticeable thickening of its external wall and the adjacent ectoderm (*L*).

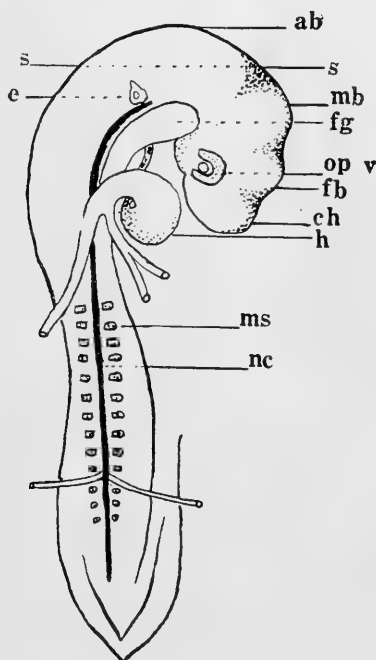
The ectoderm now begins to dip in from the outside (text-fig. 2, *B*, *l*.) and at the same time pushes the outer wall of the optic vesicle before it. There is thus formed the double-walled optic cup. This stage is reached in the chick at about the age of fifty hours. Figure 44 represents a section through the head of a chick of fifty-six hours' incubation. The invagination of the ectoderm, *Lc*, and the formation of the optic cup is very noticeable. At this age the optic vesicle is reduced to a shallow cavity and there is marked difference in the thickness of the two walls, the anterior wall being much the thicker.

The edges of the ectodermal invagination approach each other and finally unite to form the lens vesicle. Later this separates completely from the ectoderm which forms a continuous layer over it (text-fig. 2, *C*, *l*). As development proceeds the optic cup becomes deeper and deeper until the two walls (text-fig. 2, *r* and *Pg*) are in contact and the cavity of the primary optic vesicle is wholly obliterated. The inner layer (*r*) rapidly increases in thickness and finally develops into all the different layers of the retina, excepting the pigment layer which develops from the outer wall (*Pg*).

A microphotograph of a section through the head of a sixty-four-hour chick is given in figure 45. At this age the lens vesicle (*Lc*) is completely formed and separated from the ectoderm, which appears as a dark line immediately in front of and adjacent to the lens vesicle. Owing to the hardening process, the optic cup is somewhat distorted, but is seen to be well formed. The retinal portion of the cup (*R*) is several times thicker than the posterior pigment portion (*P*).

Sections in a vertical plane show that all portions of the wall of the optic vesicle do not grow at the same rate. The cells on the dorsal side multiply more rapidly than the others and soon produce an unsymmetrical shape. A diagram of a vertical section through these parts is shown in text-figure 3, *A*. This view would be obtained, were a section made through the devel-

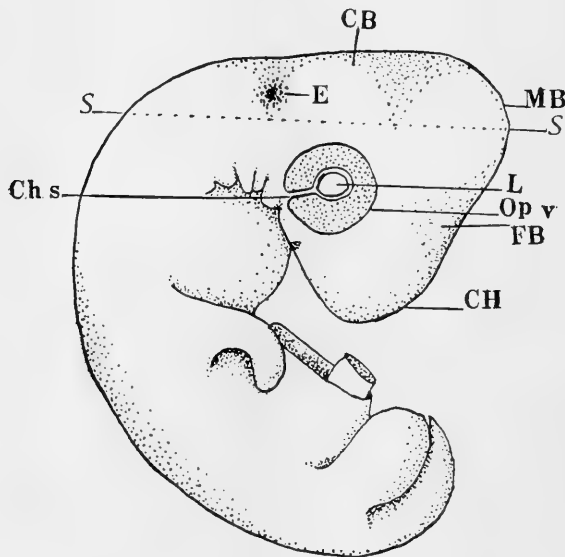
opening eye vertical to the paper and along the line *S-S* of text-figure 2, C. For the sake of convenience the ectoderm is omitted. The forward projection of the dorsal part, the retardation of the ventral part, and the almost complete obliteration of the primary optic vesicle are very noticeable.



Text fig. 4 Chick of about forty-four hours' incubation, showing the side view of the head. *ab*, afterbrain; *fg*, fore gut; *h*, heart; *ms*, mesoblastic somites; *nc*, notocord; *opv*, optic vesicle.

The retardation of growth of the ventral cells prevents the complete closing over or uniting of the ventral border of the optic cup at this stage. This space or groove which is left is the chorioid fissure. It is more clearly understood by examining text-figure 3, B, a section made at right angles to the paper and along the line *S-S* of text-figure 3, A. The ectoderm is very thin and enables one easily to perceive the lens, the horse-shoe-shaped rim of the optic cup (*opv*), and the wide chorioid fissure below.

As development advances this fissure gradually narrows by the proliferation of the cells of the walls in this region, so that by the time the chick has reached the age of ninety-six hours it is a mere cleft (text-fig. 5, *Chs*). The edges of the walls of the fissure have not only grown closer together, but the lower rim of the optic cup, which was retarded, has grown forward until the whole front of the rim is symmetrical. The walls of the chorioid



Text fig. 5 Side view of chick of about ninety-six hours' incubation. *CB*, cerebellum; *CH*, cerebral hemispheres; *Chs*, chorioid fissure; *E*, ear pit; *FB*, fore-brain; *L*, lens; *MB*, mid-brain; *Opv*, optic vesicle; *s-s*, plane of section of figure 3.

fissure unite first at the rim of the optic cup, the union continuing inward until the fissure is completely closed.

In the earliest embryo sparrow secured (figs. 1 and 16) the eye is slightly further developed than in the 100-hour chick, although the chorioid fissure (*c*) still is visible. Figure 48 is a photograph of a section through the eye at this age. The optic stalk (*OpSt*) is plainly visible. The original optic vesicle has completely closed by the apposition of the anterior and posterior walls. The cavity of the lens has been obliterated by the rapid growth of



the cells forming the posterior part of the capsule. The epithelium covers the lens, the cornea has not developed, and the anterior chamber of the eye has not yet been formed.

Since the brain arises from the ectoderm, the two layers forming the optic cup are of ectodermal origin. The uvea of the iris and the pigment of the ciliary bodies, since they are derived from the retina, are likewise ectodermal. The lens is developed directly from the ectoderm. The thin layer of ectoderm covering the embryonic lens later becomes the conjunctival epithelium. All other structures of the eye—the chorioid, iris, sclera, muscles (the muscles of the iris possibly excepted), blood-vessels, etc.—are derived from the mesoderm.

#### THE CORNEA, IRIS, AND AQUEOUS CHAMBER

For some time after the formation of the lens vesicle the front of the eye is covered only by the ectoderm, which later becomes the conjunctival epithelium of the cornea. Little change is seen in the development of the cornea, iris, and aqueous chamber of the sparrow up to the second day of incubation.

Figure 31, which represents the development at the age of two days, shows that the conjunctival epithelium (*c*) is the only portion of the cornea present and that it is applied closely to the lens. Neither the iris nor the aqueous chamber shows any development. It is true that there is an open space between the lens (*L*), conjunctival epithelium (*C*), mesoderm (*m*), and the pigment portion of the retina (*P*), but this can scarcely be considered the beginning of the aqueous chamber. A slight projection from the mesoderm (*Md*) is found. This is the beginning of the posterior layer of the cornea, or the membrane of Descemet.

Hertwig ('90) says that in the chick as early as the fourth day a thin structureless sheet of mesenchyme extends between the lens and the epidermis, into which numerous mesenchymatous cells later migrate from the margin and become the corneal corpuscles. He further states that these corpuscles later form the corneal fibers, and the structureless sheet forms the cementing substance between the fibers, the membrana elastica anterior, and the membrane of Descemet.

With three days of incubation this rather blunt projection has extended some distance farther toward the front of the lens (fig. 32). It appears as an undifferentiated mass of mesodermal cells. The conjunctival epithelium is still closely applied to the lens.

By the fourth day (fig. 33) the growth from the mesoderm has extended a considerable distance over the front of the lens, and in doing this it has pushed the epithelial layer away from the lens. The cells of this projection have become very much elongated, somewhat spindle-shaped and for the greater part are arranged in a single row. This thin projection fills only a part of the space formed by the separation of the epithelium from the lens. It lies closer to the surface of the lens than to the epithelium. No evidence of the developing iris is seen at this age.

Since this projecting mass of cells seems to be a distinct outgrowth from the mesoderm and at first is not even in contact with the epidermis, one is forced to disagree with Kessler ('77), who claims it is a product of the secretion of the epidermis, and to accept Kölliker's ('83) view that it is mesenchymatous in origin. The development of the mammalian eye also substantiates this view.

At the fifth day (fig. 34) the membrane of Descemet is a single layer of spindle-shaped cells extending over the entire surface of the lens. A space separates the epithelium from this layer of mesenchyme cells throughout its entire extent. Near the periphery this layer is somewhat thickened (due to the migration of mesenchyme cells from the periphery into it) and is several cells in width. This condition corresponds very closely to the fifteen-day chick as described by Kessler and to the fourth month in the human fetus (Wolfrum, '70'). A second slight projection of the mesenchyme, posterior to the above layer, is the beginning of the iris.

The membrane of Descemet increases in thickness by further migration of mesenchymatous cells from the periphery, and by the sixth day it forms a uniform layer throughout its extent. It is now closely applied to the epithelium (fig. 35). The spaces

which existed between this membrane and the epithelium on the one side and the lens on the other have disappeared. The membrane of Descemet now measures 0.004 mm. in thickness and the epithelium 0.016 mm., making a total thickness of 0.010 mm. The iris is still very rudimentary.

A marked difference is noticed in the cornea at the age of about seven days (fig. 36). The substantia propria (*St*) of the cornea appears as a definite layer, which is, however, not uniform in thickness. At the center of the lens it measures 0.016 mm., while at the periphery it is 0.024 mm. thick. This layer is apparently formed by an ingrowth of mesenchymatous cells between the epithelium and the membrane of Descemet. The line of junction with the epithelium doubtless represents the membrana elastica anterior described by Hertwig. These cells are still undifferentiated and are similar to those of the mesenchyme at the margins of the cornea. The iris is still rudimentary. The margin of the original optic cup, composed of its two layers (*P* and *R*), still extend to the lens. This condition has obtained from the second day up to the present age. At this stage, however, these two layers are relatively thinner than in the earlier. The pigment layer is becoming pigmented.

From this stage on to the adult eye about the only structural changes noted in the developing cornea are an increase in the thickness of the substantia propria, due to further migration of mesenchymatic cells from the periphery, and a differentiation of these cells into elongated spindle-like cells closely packed together. Table 1 gives the modifications in the thickness of the different layers of the cornea at different ages; it also gives the diameters of the eye in the axial and equatorial directions. This shows that the cornea increases in thickness from the second day of incubation until shortly after the age of hatching, when it measures 0.162 mm. in thickness. From this age on there is a gradual reduction in its thickness to the adult condition, when the total thickness measures 0.071 mm. This variation in thickness is due mainly to modifications in thickness of the substantia propria. The other layers of the cornea, the epithelium and the membrane of Descemet, also show slight but similar

changes. This increase in thickness is thus caused mainly by the proliferation of cells in the substantia propria. As these cells become differentiated into the thin lamellar-like bands, they occupy less space thus reducing the thickness.

By the eighth day the projection from the mesenchyme (fig. 37, I), destined to become the iris, extends almost to the lens. A space, bounded by the cornea in front and partially by the iris and the lens behind, is the beginning of the true aqueous chamber.

TABLE 1

*Showing the thickness of the different layers of the cornea in the developing eye and the adult. Also the axial and equatorial diameters of the whole eye at different ages. All measurements are in millimeters. H-2 and H-4, hatched two and four days; H-fl, the age of leaving the nest, or the first flight*

AGE	CONJUNCTIVA	SUBSTANTIA PROPRIA	MEMBRANE OF DESCMET	TOTAL THICKNESS	OUTSIDE DIAMETER WHOLE EYE	
					Axis	Equator
<i>days</i>						
2	0.010	0	0	0.010	0.629	0.833
3	0.016	0	0	0.016	0.748	1.200
4	0.016	0	0	0.016	0.833	1.377
5	0.016	0	0.002	0.018	1.360	1.870
7	0.016	0.016	0.004	0.036	1.495	2.080
8	0.016	0.040	0.004	0.060	2.016	2.795
9	0.016	0.054	0.004	0.074	2.405	3.185
12	0.016	0.072	0.004	0.092	3.712	4.032
H-2	0.020	0.136	0.006	0.162	4.224	4.800
H-4	0.024	0.060	0.006	0.090	5.440	5.962
H-fl	0.024	0.048	0.004	0.076	5.888	6.912
Adult	0.014	0.054	0.003	0.071	6.192	7.236

At this age, however, it is confined to this limited region and does not extend across the front of the lens. The cornea is in direct contact with the lens over most of its anterior surface.

At the ninth day (fig. 38) the iris (*I*) has grown until it is applied closely to the surface of the lens for some distance. It still consists of a thin free margin and the whole is composed of undifferentiated mesenchyme cells. This corresponds well with the condition found in the young albino mouse at the age of two days as described by Nussbaum ('12). The aqueous chamber

(Ac), which is still limited to this region, is now completely separated from the posterior cavities. The retina and pigment layers are relatively very much thinner and have begun to bend around so as to present a flat face to the surface of the lens. They are also beginning to show evidences of folding. This is an early stage in the development of the ciliary processes.

According to Kessler ('77), the first appearance of the ciliary bodies is seen in the chick on the ninth or tenth day of incubation. This is almost one-half the incubation period of the chick. According to my observations, the first appearance of the formation of the ciliary bodies in the sparrow occurs at a relatively later age of incubation. In this folding the retinal, the pigment, and the mesenchymatic portions are all involved. In man the ciliary bodies first appear, according to Kölliker ('83), at the end of the second or the beginning of the third month. Keibel and Mall ('12) state that the ciliary bodies are recognizable in the human fetus at the end of the fifth month.

The distal portion of the pigment layer in the sparrow is now well pigmented and touches the posterior surface of the developing iris, although the line of separation can still be easily seen. The mesenchymatous cells are differentiating, so that the scleral and chorioid portions are easily distinguished. Both of these layers become rapidly thinner as they are followed posteriorly from the lens. The iris now shows a clear connection with the developing chorioid and the cornea with the sclera.

Figure 39 shows the development at ten days. The cornea has increased in thickness by increase in the substantia propria. Its total thickness now measures 0.100 mm. The thickness of the different parts is: membrane of Descemet, 0.006; substantia propria, 0.074; epithelium, 0.020 mm. The iris extends farther over the front of the lens, but mainly as a continuation of the thin layer of mesenchyme. The pigmented portion of the retina is much thinner at its free margin, but posteriorly it has thickened. The retinal layer is also thinner at the free margin. The aqueous chamber is still limited to this angle.

By the twelfth day of incubation (almost the time of hatching) marked changes are noticed in the development of the iris and

ciliary bodies (fig. 41). The iris has grown only slightly farther over the lens but has been greatly thickened by increase in the number of mesenchymatous cells. Numerous blood-vessels are seen in it. The pigment of the retina, which has become closely packed together and thinner, extends almost to the free margin. The retinal portion covers the entire posterior surface as a very thin membrane. There is no evidence of the formation of muscles in the iris at this age. The retina and pigment layers near the peripheral margin of the iris have become much thickened and folded, so that the separation from the developing chorioid is scarcely discernible. In fact, these three structures seem to be more or less blended with each other in forming the ciliary bodies (*Cb*). This is due to the fact that the retinal layer, the pigment layer, and the mesenchyme each takes part in the formation of the ciliary bodies. The aqueous chamber is still limited in its extent to the region of the iris. Nothing indicative of the ciliary muscles can be noted at this age. The cells of all these structures still lack complete differentiation. The early formation of the scleral plates is indicated by a denser grouping of the cells in the more or less differentiated sclerotic coat.

By the thirteenth day (fig. 42) the cornea is beginning to separate from the lens, so that the aqueous chamber now extends completely across the lens as a thin space. This is apparently due to an increase in the curvature of the cornea. The ciliary body has become more definitely formed and the iris more typical of the adult.

Two days after hatching, all these structures are almost as perfectly formed as in the adult. The tissue, however, are not fully differentiated. The ciliary bodies are well formed. The ciliary muscles are partly developed and some few cells show slight striations. In the adult these muscles are all striated in the bird. The scleral plates are formed. The iris is similar to that of the adult, except that the muscles are not completely striated. In the adult they are all striated. The thin layer of retina back of the pigment layer of the iris is so covered and impregnated with pigment that it is not visible. It can, however,

easily be traced over the ciliary bodies as a non-pigmented layer to the periphery of the iris. The curvature of the cornea has greatly increased so that the aqueous chamber has the same shape as in the adult.

By the fourth day after hatching all these structures have reached a development similar to that of the adult except the ciliary muscles and the muscles of the iris. These are not completely striated until a few days later, when the bird is about to leave the nest.

The development of the cornea, the aqueous chamber, and the iris of the duck and the crested grebe has been studied by C. Lindahl ('15). In general, the development of these structures in the sparrow corresponds with his descriptions in these birds. Some differences are noted in the age at which certain parts appear. These I attribute to the differences in the periods of incubation of these birds. He has found similar discrepancies in the two birds studied.

We both agree that, with the exception of the epithelial layer, the cornea is derived from the mesenchyme; that the membrane of Descemet appears first and is soon followed by a gradually thickening substantia propria; that the aqueous chamber, at first confined to the angle at the peripheral portion of the iris, increases in size by an increase in the curvature of the cornea; that the iris is developed mostly from the mesenchyme, the retinal and pigment layers forming only a thin densely pigmented layer adjacent to the lens; and that the ciliary bodies are developed from the retinal and pigment layers, and from the undifferentiated chorioid, or mesenchymatous cells, the latter forming their main bulk.

The first appearance of the sphincter and dilator muscles of the iris is at about the time of hatching. They occur as denser areas in the posterior portion of the stroma. From my sections it is impossible to state whether they are mesenchymal or ectodermal in origin. According to Keibel and Mall ('12), these muscles are of ectodermal origin in man and that they take their origin from the iridal portion of the outer layer of the optic cup at about the twenty-fourth to the thirtieth week of fetal life (Heerfordt,

'00). They state that this mode of development of the dilator muscles was first observed by Grynfeldt ('99) in the rabbit, in which they were first seen at the age of fourteen days after birth. This mode of development was later verified by Nussbaum ('99) in the mouse and in birds. This view is also held by Szili ('01), Herzog ('02), Collin ('03), and Lewis ('03).

Opposed to the ectodermal origin of the muscles of the iris is the view of Hertwig ('90), who maintains that they are developed from the mesenchyme. In describing their development in mammals, he says (p. 399): "Mit der Flächenausbreitung der beiden Epithellamellen hält die ihnen von aussen anliegende Mesenchymschicht gleichen Schritt. Sie verdickt sich und liefert das mit glatten Muskelzellen und Gefässen reich versehene Stroma der Iris."

Which of these views is correct I am unable to state, as my sections throw no light on the subject. The first appearance of these muscles as more dense masses in the stroma portion of the iris rather favors the view that they are of mesenchymatous origin. It does seem strange that these muscles are almost the only ones in the whole organism not derived from the mesenchyme. The only others I know of are the smooth muscles of the glandulae sudoriferae, which Kölliker ('98), Heidenhain ('93), and Stöhr ('02) claim are of ectodermal origin. Authorities agree that the ciliary muscles are derived from the mesenchyme. Why they should be developed from mesenchymatous tissue when the conditions for ectodermal origin are just as favorable as in the iris muscles is difficult of explanation.

It is interesting to note that complete striation of the ciliary muscles and the muscles of the iris occurs but a short time before they are to function in aiding the bird in its flight. Why these muscles are striated in birds and smooth in mammals has been explained (Slonaker, '18) as due to the difference in the rate and manner of locomotion in the two groups of animals. The rapid flight of the bird requires quick changes in accommodation and in the size of the pupil which could not be accomplished by the slow-acting smooth muscle.



## THE LENS

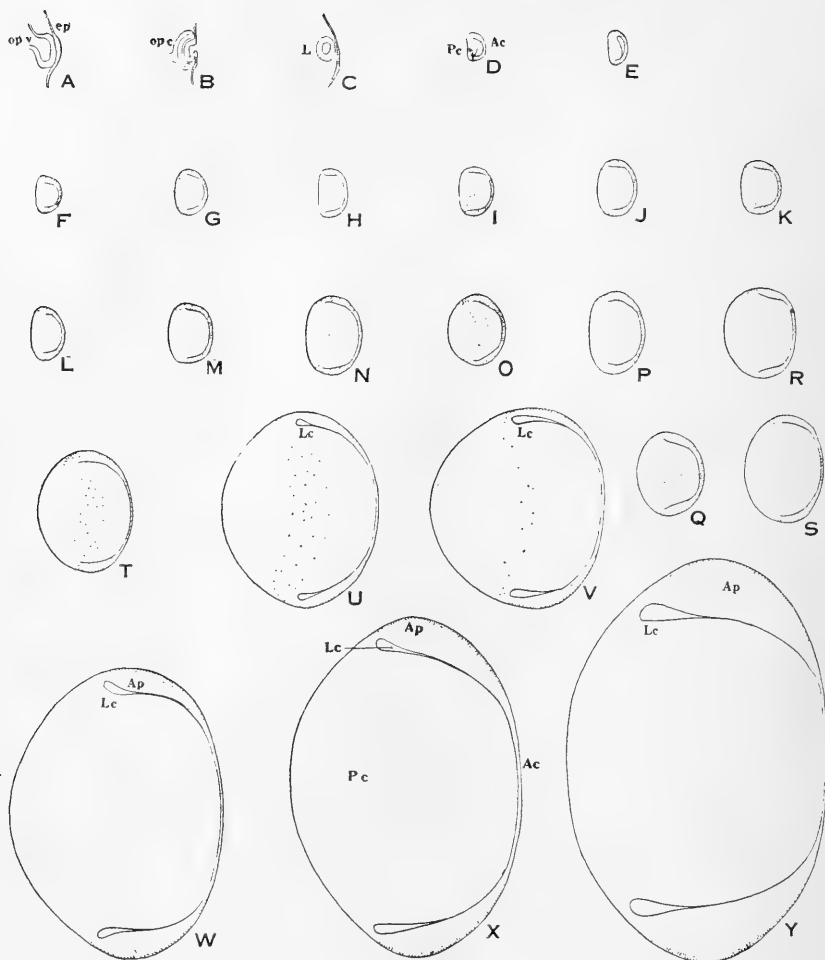
Though the development of the lens of the sparrow follows in general that of other vertebrates, some modifications are found which warrant a brief description and comparison of the different stages.

Figure 6 shows that the lens is developed from the ectoderm. This layer at first thickens (*A*) immediately over the area of contact with the optic vesicle. Rabl ('99) states that the first thickening of the ectoderm in the formation of the lens in the chick occurs at the age of twenty hours' incubation. Other authors say it varies between eighteen and twenty-one hours or more. Froriep ('06) concludes that it occurs in the chick at the end of the second or the beginning of the third day. A very marked thickening is seen in the forty-hour chick (fig. 43, *L*).

Keibel and Elze ('08) show a recognizable thickening epithelial plate, the beginning of the lens, in a 4-mm. mammalian embryo. This corresponds to the twenty-hour chick embryo.

This thickened area soon becomes concave exteriorly and sinks in to form a pit. This occurs in the chick (Duval, '89) when 5 to 7 mm. long, with 22 to 25 somites at the age of forty-six to fifty-two hours' incubation. Text-figure 6, *B*, and figure 44, *Lc*, show this invagination of the epithelium in the chick at the age of fifty to fifty-six hours' incubation. This is at a slightly later age and shows that the edges of the depression are beginning to approach each other to form the lens vesicle. With this invagination, the wall of the optic vesicle in contact with this area is apparently pushed inward, gradually obliterating its cavity, and finally forming the double-walled optic cup.

It has been experimentally demonstrated by Spemann ('01) and Lewis ('04) that the optic cup is not formed by pressure exerted by the developing lens. They have shown that when the optical vesicles are implanted in abnormal situations the optic cups will be formed without the development of a lens. They also conclude that the formation of the lens is due to the stimulus caused by the optic vesicle's coming in contact with the ectoderm. This stimulus will cause any portion of the ectoderm brought in contact with the optic vesicle to form a lens. The formation of



Text fig. 6 Drawings of the developing lens from the early stages to the adult, showing the rapid increase in thickness of the posterior wall of the lens vesicle and relative lack of growth of the anterior wall. The distribution of the nuclei at these various ages is represented by the dotted areas. The anterior portion of each drawing is to the right. Drawings A to S, inclusive, refer to ages of incubation; T to Y, after hatching. *Ac*, anterior wall; *Ap*, annular pad; *ep*, ectoderm; *L*, lens vesicle; *Lc*, lenticular chamber; *opc*, optic cup; *opv*, optic vesicle.

A, chick 40 to 48 hours; B, chick 56 hours; C, chick 64 hours; D, chick 68 hours; E, chick 90 to 100 hours; F, sparrow 2 days; G, sparrow 2½ days; H, sparrow 3 days; I, sparrow 4 days; J, sparrow 5 days; K, sparrow 6 days; L, sparrow 7 days; M, sparrow 7½ days; N, sparrow 7¾ days; O, sparrow 8 days; P, sparrow 9 days; Q, sparrow 10 days; R, sparrow 11 days; S, sparrow 12 days; T, sparrow at hatching; U, sparrow nestling 2 days; V, sparrow nestling 4 days; W, sparrow nestling about 8 days; X, sparrow fledgling just flying; Y, adult sparrow.

the lens is, therefore, not confined to the region of the ectoderm where normal development occurs, but to any area with which the optic vesicle comes in contact. Froriep ('06) concludes that the formation of the optic cup is due not so much to the invagination, but to a more rapid outgrowth of the rim.

At this age there is no space between the invaginating epithelium and the anterior wall of the optic cup. A slight remnant of the cavity of the optic vesicle is still seen between what is now the outer and the inner (invaginated) layers of the optic cup. As the edges of the pit approach each other, a space appears between the forming lens and the inner layer of the optic cup. This is due to the ingrowth of mesenchymatous cells and the formation of the vitreous body to be described later. By the complete growing together of the edges of this pit the lens vesicle is formed (text figure 6, C, and figure 45, *Lc*). This occurs in the chick at about sixty-four hours' incubation. Froriep ('06) says that it varies between sixty-two and seventy-three hours. The shape of the lens vesicle is now almost a sphere. The axial and equatorial diameters are 0.128 mm. and 0.144 mm., respectively.

After the complete closure of the lens vesicle, it remains in cellular contact with the epithelium for a short time. This connection very soon disappears and the epithelium extends across and immediately in front of the vesicle in a single layer of cells which later becomes the conjunctival epithelium (text fig. 6, C).

At this stage the wall of the lens vesicle in the chick is almost uniform in thickness. A slight difference, however, is noticed. The thickness of the anterior wall is 0.04 mm. and that of the posterior wall is 0.055 mm. (Froriep). In the pig at this stage of lens development I find the anterior wall is decidedly thinner, measuring 0.04 mm., while the posterior measures 0.072 mm.

About this time, according to Keibel and Mall ('12), the lens capsule is seen. They say it is developed from the cells of the lens and not from the surrounding mesoderm.

In the 6-mm. pig embryo, which represents approximately this stage, I find a stream of mesenchymatic cells migrating into the optic cup between its edge and the lens vesicle. The

greater mass of these cells is located near the posterior axis of the lens. These cells are arranged with their long axes parallel to the surface of the lens. The thin stream of cells connecting this mass with the outside mesenchyme is closely applied to the lens and is composed of a single layer of cells whose long axes are also arranged parallel to the surface of the lens. This is probably the beginning of the capsula vasculosa lentis, as well as of the cells from which the vitreous body is developed. In the sparrow eye I have seen no evidence of the capsula vasculosa lentis.

Very soon after the complete formation of the lens vesicle the cells of the posterior wall begin to elongate and to extend anteriorly into the lumen of the vesicle. This is seen in text-figures 6, D and figure 46, which represent the condition of the chick at the age of sixty-eight hours' incubation. This growth is not uniform, but the cells in the axial region grow the fastest and produce a convex inner surface. There is thus a gradual transition from the long, fiber-like cells in the center of this protuberance to the short cells at the equator of the lens. In mammals the anterior wall of the vesicle is of uniform thickness and becomes in the adult a very thin layer called the lens epithelium. In birds and reptiles the equatorial part of this layer behaves in a different manner. It develops into the 'Ringwulst,' or what I have designated the annular pad. This will be described later.

The posterior surface of the lens vesicle is now almost flat. The thickness of the anterior wall is 0.036 mm. and the posterior wall 0.096 mm. The axial and equatorial diameters have relatively changed, giving a more lenticular shape to the lens. The axial diameter is now 0.176 mm. and the equatorial 0.232 mm.—a ratio of 1:1.3.

In the four-day chick (fig. 47) the cavity of the lens vesicle is almost obliterated. The change of the shape of the lens is very noticeable. The axial diameter is 0.160 mm. and the equatorial 0.288 mm.—a ratio of 1:1.8. The equatorial diameter has increased much more rapidly than the axial diameter, apparently because of the great increase in the number of cells of the posterior wall.

The cells of the posterior wall continue to grow in length, to become more fiber-like, and to push forward the inner surface of the wall until the cavity of the vesicle is completely obliterated by the union of the posterior and anterior walls. This occurs in the chick, according to Froriep ('06), in the second half of the fifth day of incubation. At this age he gives the following dimensions: axial diameter, 0.29 mm.; equatorial diameter, 0.57 mm.; thickness of anterior (epithelial) wall, 0.018 mm.; posterior wall, 0.272 mm. The ratio of the two diameters is now about as 1:2. The lens has thus changed greatly in shape from the almost spherical form found in the early stage of the lens vesicle to the lenticular shape seen when the cavity of the lens vesicle has been completely obliterated. In other words, the axial and equatorial diameters have changed from approximately a 1:1 to a 1:2 ratio, respectively.

According to Rabl ('00), in man, after the lens fibers have reached a length of about 0.18 mm., they no longer divide, but simply grow in length. An increase in the number of lens fibers occurs at the junction of the anterior epithelial layer with the posterior portion near the equator.

If the same be true in the development of the lens of the pig, this length of lens cells is found in the 11-mm. embryo. This corresponds very closely to the stage of the sixty-eight-hour chick when these cells are 0.076 mm. long. My sections do not show whether mitotic division is still occurring in these cells. It appears, however, that cell division is taking place more rapidly at the periphery than at the center of this protuberance. After complete obliteration of the lens vesicle, there is no doubt that in the sparrow new lens fibers are formed near the equator of the lens at the junction of the lenticular part with the epithelial layer.

The development of the eye has now reached a stage which corresponds very well with the earliest age secured of the sparrow. This was at about two or two and one-half days' incubation. If the rate of development is proportionally the same in the two birds, the first stage secured in the sparrow is between two and three days. At this age all that is left of the original

lens cavity is represented by the line of junction of the anterior and posterior walls (text-fig. 6, F and figure 48). This line of junction is noticeable in all ages of the sparrow studied. Even in the adult it is very conspicuous, especially in the equatorial region.

A noticeable difference in the shape of the lens of the sparrow and that of the chick, occurs at this stage of development; the sparrow lens is more spherical. The axial and equatorial diameters in the sparrow form a ratio of 1:1.31, while in the chick the ratio is approximately 1:2. The sparrow lens is more nearly spherical, not only at this stage, but this condition obtains throughout life. When the similar dimensions are compared in the adults, the ratio in the sparrow is 1:1.56 and in the hen 1:1.73. These ratios vary with different species of birds. In the seventeen different species which I have measured the ratio of axial diameter to equatorial has ranged from 1:1.83 in the bluebird (*Sialia sialis*) to 1:1.28 in the least sandpiper (*Pisobia minutilla*). In the fox sparrow (*Passerella iliaca*) the ratio is 1:1.58. The average ratio of all these measurements is 1:1.58, which is practically that of the English sparrow.

From this time on to complete development of the lens the most noticeable change is that of size. This is due almost wholly to an increase in the lenticular portion by the addition of new cells formed at the equator and applied to the surface of the lenticular core in a parallel manner, thus forming successive layers. These cells are long, six-sided, flattened structures which extend from the anterior to the posterior surface. The only part the anterior layer takes in increasing the size is at the equator, where it is modified into the annular pad.

The posterior surface shows the flattened appearance described in the chick. This condition persists until about the eighth day, when the anterior and posterior surfaces have about the same curvature (text-fig. 6, O). This relation of the curvature of the two surfaces remains until about the age of hatching (text-fig. 6, T), when the posterior surface has the greater curvature. This condition persists throughout life.

Accompanying this change in relative curvature of the two surfaces, there is also a change in the ratio of the length of the axis to the equatorial diameter. Table 2 shows that there is an increase in the ratio from an almost 1-to-1 relation in the two-day stage to a 1-to-1.56 ratio in the adult. In other words, there has been a gradual change from the slightly flattened spherical shape to the lenticular form. It also shows that the increase in equatorial dimension is due to the development of the annular pad rather than to much change in the ratio of the axial and equatorial diameters of the lenticular portion.

In the two-day embryo the nuclei of the cells of the anterior layer are scattered throughout the layer, while those of the posterior layer are situated about midway of the length of the cells. The position of the nuclei is represented by the dotted areas in the drawings of text-figure 6.

As the lens increases in size, the thickness of the anterior layer remains practically the same. In other words, the increase in size of the lens is largely due to the growth in length and increase in number of the cells of the posterior layer, but the nuclei of the lenticular portion become more and more inconspicuous until they have almost disappeared by the twelfth or thirteenth day (text-fig. 6, T), the age of hatching. Two days after hatching (U) the lenticular part is almost entirely free from nuclei, except a few cells which join the annular pad. They have either disappeared or have changed so as no longer to take the stain. The nuclei of the anterior layer are conspicuous until about the time the bird leaves the nest. Clear and unobstructed sight can therefore not be possible in the sparrow until about the age of flying.

A better idea of the relative growth of the different parts of the lens can be obtained from the following table of measurements. All measurements were made with the eyepiece micrometer and of sections as nearly as possible through the center of the lens, along the axis of vision.

This table shows almost a uniform increase in the dimensions of the different parts. Some variations occur, especially in the dimensions of the annular pad and the anterior layer. These

TABLE 2  
*Showing the progressive increase in the dimensions of the various parts of the developing lens from the beginning to the adult stage. Measurements are in millimeters*

	AGE	TOTAL AXIAL DIAMETER OF LENS	TOTAL EQUATORIAL DIAMETER OF LENS	RATIO OF EQUATORIAL DIAMETER TO AXIAL DIAMETER	AXIAL DIAMETER OF LENTICULAR PART	EQUATORIAL DIAMETER OF LENTICULAR PART	RATIO OF AXIAL DIAMETER TO EQUATORIAL DIAMETER	EQUATORIAL THICKNESS OF ANTERIOR LAYER PART	AXIAL THICKNESS OF ANTERIOR LAYER
Chick embryo.....	64 hours	0.1280	0.1440	1:1.12	0.0550				0.0400
	68 hours	0.1760	0.2320	1:1.32	0.0960				0.0360
	100 hours	0.1600	0.2880	1:1.80	0.1040				0.0340
	4½ days¹	0.2900	0.5700	1:2 —	0.2720				0.0180
	2 days	0.2590	0.3510	1:1.31	0.2360	0.2630	1:1.11	0.0440	0.0230
Sparrow embryo.....	3 days	0.2700	0.4320	1:1.6	0.2686	0.3380	1:1.26	0.0470	0.0277
	4 days	0.3240	0.5022	1:1.55	0.2957	0.4042	1:1.37	0.0490	0.0283
	5 days	0.3960	0.6120	1:1.54	0.3660	0.5080	1:1.38	0.0520	0.0300
	7¼ days	0.4140	0.6300	1:1.52	0.3940	0.5343	1:1.35	0.0477	0.0200
	7½ days	0.4950	0.7020	1:1.42	0.4750	0.5980	1:1.26	0.0520	0.0200
	8 days	0.5680	0.7740	1:1.36	0.5480	0.6540	1:1.19	0.0600	0.0200
	9 days	0.5760	0.7850	1:1.36	0.5560	0.6346	1:1.14	0.0752	0.0200
	10 days	0.7020	0.9180	1:1.31	0.6860	0.8076	1:1.17	0.0552	0.0160
	11 days	0.7200	0.9320	1:1.3	0.7040	0.8240	1:1.17	0.0540	0.0160
	12 days	0.9000	1.1500	1:1.28	0.8880	1.0300	1:1.16	0.0600	0.0120
Sparrow hatched.....	13 days	0.9360	1.1880	1:1.28	0.9240	1.0074	1:1.09	0.0903	0.0120
	2 days	1.5150	1.8700	1:1.23	1.1395	1.7700	1:1.55	0.1000	0.0120
	4 days	1.5320	2.0250	1:1.32	1.5200	1.8930	1:1.24	0.1320	0.0120
	8 (?) days	1.6842	2.2105	1:1.31	1.6722	1.9725	1:1.18	0.2380	0.0120
	Just flying	1.7894	2.7843	1:1.55	1.7794	2.4243	1:1.36	0.3400	0.0100
Adult		2.0810	3.2526	1:1.56	2.0740	2.7256	1:1.31	0.5270	0.0070

¹ Forriep ('06).



may be due to individual variation, to different effects of the reagents, or, more likely, to possible differences in the plane of section. The column giving the thickness of the anterior layer shows, after a slight increase, a progressive thinning. It is interesting to note that with this reduction in thickness the nuclei become less and less conspicuous. They either disappear (which would explain the thinning) or are rendered transparent.

The annular pad increases in thickness by the lengthening of its cells. These cells are arranged radially at first and remain so until after the beginning of the lenticular chamber (text-fig. 6, *Lc*). Soon after hatching the cells begin to show globular projections from their inner ends as in the adult (Slonaker, '18). The accumulation of these globules seems to exert a pressure between the lenticular portion and the annular pad. By the second day after hatching the lenticular chamber begins to appear slightly posterior to the middle of the annular pad (text-fig. 6, *U*). This occurs at the line of junction of the two portions of the embryonic lens. Separation occurs here because it is the place of least resistance. The cells showing the most prominent globular projections are adjacent to the forming chamber. All of these cells are still straight and converge toward the center of the lens.

On the fourth day after hatching (*V*) the cells of the annular pad have become longer and are beginning to curve posteriorly. The lenticular chamber has increased and more cells show globular secretions or projections. The lenticular chamber is apparently formed by the accumulation of this secretion. Since, in its formation, it follows the line of junction of the two formative layers, it may be said to correspond, in position at least, to the original lens cavity. With increasing age these cells become longer and more bent. Their nuclei remain in the peripheral ends, leaving the inner portions of the cells filled with granules. The lenticular chamber increases in size and seems to lag behind as the center of the lens grows forward. This anterior growth of the central part may in part be the cause of the bending of the cells of the annular pad.

The cells in the thickest portion of the annular pad are longest. The nuclei of these cells are arranged in a double row in the outer ends near the lens capsule. From this region they become gradually shorter, both anteriorly and posteriorly, and are arranged in a single layer and finally disappear from view. Toward the posterior portion of the annular pad the cells become gradually shorter and less granular until they have completely lost their identity as annular pad cells, at about the posterior border of the lenticular space. The nuclei are conspicuous in this region. A little farther back these cells gradually become longer, their nuclei disappear, and they merge into the true lens cells. There is, therefore, no sharp line of division between the cells forming the annular pad and those of the lenticular portion of the lens.

Judging from the behavior and appearance of the cells of the annular pad, both in the developing lens and in the adult, I have concluded ('18) that their function is that of nourishment. They can certainly play no part in accommodation.

By the time the young leaves the nest (X) the nuclei of the anterior layer have disappeared and the layer has been reduced in thickness, almost to the adult condition. The size of the lens is, however, considerably less than that of the adult (Y). All of the structures of the adult lens are well formed, though not completely developed. They are, however, in such a state of perfection as to give the bird distinct vision.

#### THE VITREOUS BODY

The manner of the formation of the vitreous body in the vertebrates is one of the most difficult problems. There are a number of opposing views in regard to its origin. Kessler ('77) considers the vitreous body a transudate, secreted from the adjacent loops of blood-vessels and that the cells are merely the migrated white corpuscles. Kölliker ('83), Hertwig ('90), and others consider it an exceedingly watery connective tissue which later becomes surrounded by the hyaloid membrane. Keibel and Mall ('12) claim that it is both ectodermal and mesodermal in origin; that the retinal portion of the optic cup secretes the primitive portion

and that, later, the mesodermic portion is formed. They are not clear as to just what takes place at this time.

In mammals there is a migration of mesodermal tissue into the optic cup, accompanied by blood-vessels which doubtless play an important part in the formation of the vitreous body. This was noticed in the 6-mm. pig embryo at a time when the lens vesicle had just been formed. The artery lentis, which is a branch of the *arteria centralis retinae*, finally branches profusely over the posterior surface of the lens and forms the *capsula vasculosa lentis*. These vessels appear in the human embryo about the second month of fetal life and disappear about the seventh month.

In the bird I have found no migration of mesodermal cells nor of blood-vessels as described in mammals. I have seen no evidence of a *capsula vasculosa lentis*. All that I have been able to see in my sections is the progressive formation of the vitreous chamber which is filled with a clear colorless substance. The bird does not possess an *artery centralis retinae* like that of mammals. Neither does it have an *arteria lentis* (Froriep). The internal structures of the bird eye are apparently nourished from the blood supply to the pecten. Since the pecten is developed rather late in the formation of the eye, it would appear that the vitreous body is developed as a clear secretion from the retinal layer of the optic cup, or from the mesodermic cells adjacent to the choroid fissure and rim of the cup, or possibly from both these sources.

The first appearance of the vitreous chamber occurs in the bird (chick) soon after the beginning of the invagination of the optic vesicle to form the optic cup at about the time of the complete closure of the lens vesicle. This is seen in the sixty-four-hour chick embryo (fig. 45). The substance filling this space appears clear and structureless. It contains no visible mesoderm cells or blood-vessels. The only change noted in further development is a relative increase in size as the whole eye grows larger. Later, the very vascular pecten is developed, but nothing else of a structural character have I been able to make out in the vitreous chamber during the complete formation of the eye.

## THE CHORIOID AND SCLEROTIC COATS

At the age of two days' incubation the sparrow eye shows a condensation of the mesoderm just outside of the pigment layer (figs. 48 and 49). Most of these cells are undifferentiated. Immediately in contact with the pigment layer are a number of modified, spindle-shaped cells, with large nuclei, their long axes parallel with the pigment layer. A row of these cells, one cell deep, is rather definitely arranged next to the pigment layer and extends almost to the periphery of the pigment layer. Some blood-vessels occur just outside of and in close relation to this layer. This row of cells and the adjacent blood-vessels constitute the developing chorioid.

Other spindle-shaped mesodermal cells are arranged parallel to and just outside the blood-vessels of the chorioid. They are most pronounced and differentiated at the optic axis, and they grow rapidly less distinct toward the periphery. They wholly disappear at about one-third the distance to the margin of the optic cup. The innermost portion of these cells will form later the outer boundary of the chorioid coat; the remaining cells represent the early stage in the developing sclerotic coat.

With three days of incubation the cells of the chorioid have become more numerous, especially in the region of the axis (figs. 50 and 51), and, with the adjacent blood-vessels, form a more definite layer. The formative cells of the sclerotic coat can now be traced fairly well to the lens. The cells are more definite in outline. This is especially true at the axis where they are most numerous.

By the fourth day the cells constituting the chorioid and sclerotic coats have become conspicuous and rather sharply marked off from the surrounding loose mesodermic structure (fig. 52). The combined measurement of these two coats at the axis is 0.08 mm. and at the equator 0.0024 mm. One day later, the maximum thickness at the axis is 0.108 mm. (figs. 53 and 54).

The part which these layers take in the formation of the cornea, iris, ciliary bodies, etc., has already been described under the heading, the cornea, iris, and aqueous chamber.

At the age of seven days (fig. 56, pl. 8, and pl. 9) the blood-vessels of the chorioid are more definite and form a fairly continuous open network, 0.004 mm. thick at the equator and 0.008 mm. at the axis. Its outside boundary is irregular, due to a lack of uniformity of the scleral border. The parallel cells of the sclerotic coat now form a fairly definite layer which measures 0.020 mm. at the equator and 0.028 mm. at the axis. No differentiation of these cells into cartilage and fibrous portions can be discerned. Neither is there any pigment in the cells of the chorioid.

In the nine-day embryo (fig. 69) the chorioid shows little advancement, except an increase in the vascular part. The sclera, however, shows marked differentiation. A dense mass just outside the chorioid now appears as embryonic cartilage. This cartilage-like mass of cells is thicker in the axial region and thinnest at its margins near the ora serrata. On either side of these cartilage-forming cells are spindle-shaped cells which will later form the remaining portion of the sclerotic coat. At the anterior end of the cartilage-like mass the cells are more closely arranged in an elongated slender portion which is a very early stage in the formation of the scleral, or bony plates.

Little change is noticed in the chorioid of the ten-day embryo (figs. 70 and 71). The blood-vessels are much more abundant near the pigment layer of the retina than in the outer portions of the chorioid. The cartilage portion of the sclerotic coat is more sharply marked off from the other part. The cells forming the remaining part are packed closer together into an apparently firm mass. The thickness of these two coats at different ages described is shown in table 3. In the early ages these structures were too indefinite to measure accurately; no measurements are therefore tabulated for them. Although differentiation is most pronounced in the axial region and decreases toward the periphery, the table shows that these coats are thicker at the ora serrata than at the axis at about this age. This is mainly due to the closer arrangement of the cells in the axial region.

About the age of hatching (fig. 75, *F*, and figs. 78 and 79, *F*) the first modification of the chorioid, the precursor of the area

and fovea centralis, is observed at the optical axis. It appears as a local thickening of the chorioid at the axis, and is distinct from its general increase in thickness over the back of the eye. This increase is due to a greater number of blood-vessels. (See development of the fovea.) The formative cells of the scleral

TABLE 3

*Showing the thickness of the different layers of the cornea, and the chorioid and sclerotic coats in the developing and the adult eyes. Also the axial and equatorial diameters of the whole eye at the same ages. 2 to 12 represent the ages in days of the embryos used; H-2, H-4 and H-fl represent, respectively, hatched two days, four days, and just flying. All measurements were made in millimeters and as nearly at right angles to the surface as possible*

AGE	CONJUNCTIVA	SUBSTANTIA PROPRIA	MEMBRANE OF DES- CEMET	TOTAL THICKNESS OF CORNEA	AT AXIS			AT ORA SERRATA			AXIL DIAMETER OF EYE	EQUATORIAL DIAMETE- R OF EYE
					Chorioid	Sclera		Chorioid	Sclera			
						Cartilage	Fibrous		Cartilage	Fibrous		
<i>days</i>												
2	0.010			0.010							0.629	0.833
3	0.016			0.016							0.748	1.200
4	0.016			0.016							0.833	1.377
5	0.016		0.004	0.020							1.360	1.870
7	0.016	0.006	0.004	0.026							1.495	2.080
7½	0.016	0.016	0.004	0.036							1.560	2.245
8	0.016	0.040	0.004	0.060	0.008		0.024				2.015	2.795
9	0.016	0.054	0.004	0.074	0.018		0.022				2.405	3.185
10	0.014	0.054	0.004	0.072	0.012	0.012	0.004	0.016	0.016	0.044	2.827	3.380
12	0.016	0.072	0.004	0.092	0.015	0.016	0.004	0.008	0.016	0.028	3.712	4.032
H-2	0.020	0.136	0.006	0.162	0.012	0.012	0.012	0.024	0.032	0.020	4.224	4.800
H-4	0.024	0.060	0.006	0.090	0.016	0.012	0.012	0.032	0.012	0.024	5.440	5.952
H-fl	0.024	0.048	0.004	0.076	0.040	0.040	0.020	0.024	0.024	0.008	5.888	6.912
Adult	0.014	0.054	0.003	0.071	0.134	0.043	0.004	0.016	0.020	0.012	6.192	7.236

plates appear more dense. The chorioid coat is still without pigment.

Two days after hatching (figs. 82 and 83) pigmentation of the chorioid has begun, more prominently near the lens than at the back of the eye. In this respect it corresponds to the pigmentation of the pigment layer of the retina which begins in the region of the lens at a much earlier age (see development of the retina).

The pigmentation appears as long, slender, branching strands that have no connections with other strands. Some are short and devoid of branches and others much extended and branched, anastomosing with branches from other strands. This tends to give the fenestrated arrangement of the pigment in the adult. The spaces intervening are filled mainly with blood-vessels. At this age the cornea, iris, and aqueous chamber have practically the form of the adult. The ciliary muscles are partly developed, some of the cells showing striations.

The scleral plates can now be discerned with a fairly sharp outline. They, however, still lack complete formation. The cartilage layer is now composed of true cartilage, as in the adult. It is thickest at and near the anterior margin. A much thicker mass of cartilage forms a ring around the optic nerve entrance. The thickest part of this ring measures 0.072 mm. At a distance of 0.850 mm. from the nerve entrance the cartilage is reduced to 0.020 mm. It thins out much more abruptly on the anterior side of the nerve. The cells forming the fibrous portion of the sclerotic coat are more densely arranged than in the earlier ages.

Four days after hatching (pl. 15 and figs. 92 and 93) the pigmentation of the chorioid has become more branched and dense, resembling that of the adult. The scleral plates are well formed, but do not extend backward to overlap the anterior portion of the cartilage of the sclerotic coat as in the adult. The ciliary bodies and muscles have about the normal adult appearance.

From this time on to the adult condition little change is noticed, other than completion of the development of the structures already noted. The chorioid becomes more pigmented and vascular. The outer portion of the sclerotic becomes more condensed and firm. The scleral plates change to true bony tissue and extend backward to overlap the margin of the cartilaginous portion of the sclerotic coat. Some changes in thickness are also noticed. These are tabulated in table 3.

## THE PECTEN

The development of the pecten is closely associated with similar changes taking place in the chorioid. It first appears in the seven-day sparrow embryo, a relatively late age when compared to the chick embryo where the first appearance is in the fifth day of incubation (Froriep). In cross-section it appears as a small mass of cells, accompanied by a blood-vessel, projecting from the region of the optic nerve entrance directly into the vitreous body (fig. 60, *P*). This appearance is misleading, for, as Nussbaum ('12) has shown in those animals with a pecten, the arteria hyaloidea does not enter the eye at the level of the optic disc, but through the chorioid fissure nearer the ora serrata.

The structure now consists of an elongated mass of undifferentiated cells, closely resembling those of the mesoderm surrounding the eye with which it is more or less connected. A small loop of a blood-vessel is carried in with these cells. In other words, the cells of the mesoderm seem to be flowing into the eye chamber, apparently through the chorioid fissure. In this case it would occur without involving the walls of the optic cup in any way. Some of the undifferentiated chorioid cells may be included in this migration, but there is no evidence in the sections at hand of any of the retinal cells' taking any part in the formation of the pecten. The migrating cells and the retinal cells are quite different in appearance and in their ability to take the stain. The hyaloid membrane is pushed inward by these migrating cells. If this membrane is considered a part of the retina, then, to this extent, the retina plays a part in the formation of the pecten, for the hyaloid membrane completely envelops the whole structure in the adult. In my opinion, the pecten is formed from the mesoderm, and I cannot therefore agree with Denissenko ('80), who claims that the pecten is formed by the pushing in of the retina. In one of my specimens, due to an artefact, a fold appeared in the retina. The developing chorioid and pigment layers, together with some mesodermal cells, extended into this fold. Since this happened to occur at the optic disc, and the shape closely resembled that



of the developing pecten, one might consider it normal. Similar folds in the retina do occur at any place and are mere artefacts due to the hardening process.

This view is also held by Froriep as true in the chick. He says that the first appearance of the pecten is in the chick of five days' incubation. It consists of the axial blood-vessel, surrounded by mesoderm, which enters through the chorioid fissure to form a flat roll in the groove formed by the closure of the walls of the fissure.

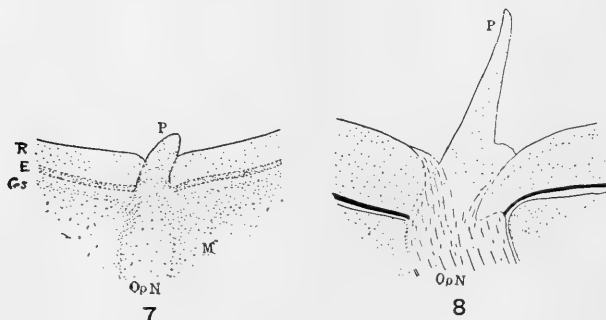
At the time of the first appearance of the pecten in the sparrow the chorioid and sclerotic layers are still in a formative stage. Blood-vessels can be seen in what is destined to become the chorioid, but as yet no definite layer is formed. Also the mesodermal cells just outside these blood-vessels are becoming elongated and will later form the sclerotic coat. At this stage of development, therefore, there is nothing to resist the passage of the mesodermal cells along the groove of the optic stalk into the optic cup through the chorioid fissure.

No differentiation of these cells in the pecten can be seen at this age. They must consist, however, of cells similar to those which are forming the chorioid, blood-vessels, etc., since they have a common origin and closely resemble them.

A few hours later in the developing pecten this mass of cells has increased considerably in volume. In cross-section the pecten now appears as a short, blunt cone. Figure 61, *P*, and text-figure 7, *P*, illustrate the condition of development at the age of seven and three-quarter days. The mesodermal cells are extending into the conical projection. At this age all of these cells are undifferentiated and resemble the typical embryonic mesoderm. They are evenly distributed through the mass, except at the base where they are less numerous in the center. There are a few blood-vessels and blood-cells at the base, near the optic disc.

The appearance of the pecten as a cone is deceptive and is due to the sectional view. As a matter of fact, it is more like a flattened cone or ridge whose base is elongated and curved to fit the inner curvature of the eye and the closing chorioid fissure.

The elongated base at this age measures 0.390 mm. long and 0.187 mm. broad, and the cone extends 0.102 mm. into the vitreous body. This corresponds closely to the condition in the six-day chick as described by Froriep. He says that at this age this roll has widened and extends into the vitreous chamber as a thick leaf or ridge. The large blood-vessel which remains in the base of this ridge is later called the pectineal artery. It sends smaller vessels up into this flat projection where they are most numerous at the margin or free edge. The ridge is attached to the wall along the fissural groove.



Text fig. 7 Enlarged diagrammatic drawing of a cross-section of the early stage of the developing pecten as seen in the seven-day sparrow embryo. *C-S*, developing chorioid and sclerotic layers; *E*, pigment epithelium; *M*, mesoderm; *P*, pecten; *OpN*, optic nerve; *R*, retina.

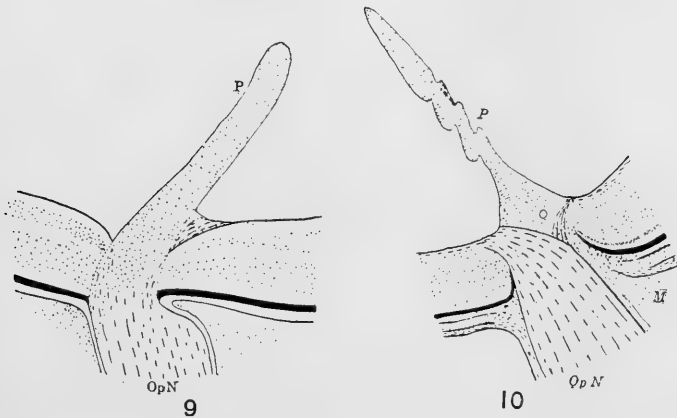
Text fig. 8 Enlarged diagrammatic drawing of a cross-section of the developing pecten in the eight-day sparrow embryo. *OpN*, optic nerve; *P*, pecten.

At the age of about eight days this flat projection has grown much farther into the vitreous body, and has become thinner. The cells still show no differentiation and are uniformly distributed throughout the main bulk of the growth. This is shown in text-figure 8, *P*, and figure 67, *P*. Spindle-shaped cells are seen in the optic-nerve region. Among these are scattered numerous cells similar to those forming the pecten.

By the twelfth day of incubation the pecten has greatly increased in dimensions. Its earlier broad form has been changed to a flattened, plate-like mass of almost uniform thickness (text-fig. 9, *P*, and fig. 76, *P*). Its length is now 0.8650 mm.

and its thickness 0.1142 mm. The cells are more closely arranged forming a rather compact layer near the surface of the pecten. These cells also show some granular bodies around their periphery, which, at a later age, become pigmented and form the pigment of the pecten.

The first indication of folding into plaits is seen at the age of thirteen days, or the age of hatching (text-fig. 10, *P*). The folds appear as three slight undulations about the middle of the pecten. At this place the thickness has been somewhat



Text fig. 9 Enlarged diagrammatic drawing of a cross-section of the developing pecten in the twelve-day sparrow embryo. *OpN*, optic nerve; *P*, pecten.

Text fig. 10 Enlarged diagrammatic drawing of a cross-section of the developing pecten in the sparrow at the age of hatching. The first indication of folds is seen. *M*, mesoderm; *OpN*, optic nerve; *P*, pecten.

reduced. It is still, however, many times thicker than the fully developed pecten. This condition corresponds to the ten-day stage in the chick as described by Froiep. He says that the pecten has become much thinner and begins to show slight indications of the folds. The pectineal vein lies at the base of the pecten in the optic-nerve fibers. Lieberkühn ('72) says that in the twelve-day chick embryo this vein leaves the pecten at its distal end. Later in the sparrow the pecten extends its base farther toward the ora serrata, so that in the adult, the veins leaves the pecten slightly distal to the center of its base.

During the two days following hatching very rapid changes occur in the pecten. At two days of age it has ten folds and has become almost as thin as the adult pecten (fig. 84, P). Four days after hatching the number of folds has increased to eighteen. At this age the first pigment granules appear. They are scattered in the tissue near the surface of the tip or free margin, which becomes in the adult the crest.

At the age of six days after hatching the pecten has practically reached the form of the adult (fig. 93, P). The twenty folds, the number in the adult eye, can be easily distinguished. The pigmentation, however, is not complete.

There is quite a difference in the relative time of the appearance of the folds of the pecten in the chick. Nussbaum ('01) finds the number in the eleven-day chick embryo to be seven and in the thirteenth-day to be seventeen. Kessler ('77) found, in the twelve-day chick, fifteen distinct folds, the middle one being the longest. In the seventeen- or eighteen-day embryo he says the folds are the same in number as in the adult. Huschke ('35) gives the number of folds in the hen as seventeen.

As stated above, the pecten in the six-day sparrow has reached the adult condition, with the exception of its pigmentation. Pigment granules were first seen in the crest of the four-day sparrow. At the sixth day the crest is more densely pigmented and there has been a progressive pigmentation from this region down the angles of each fold toward the base. This is most pronounced near the crest, gradually grows less toward the base, and disappears at about one-third the distance from the crest to the base. Everywhere the pigment appears as small granules which have a uniform and even distribution. The greater pigmentation of one region over another is thus due to a closer arrangement of these granules.

This spreading continues, and by the tenth day after hatching a very few pigment granules are found in the proximal region. In the meantime the granules have become more numerous in the other portions, and, in the crest, have begun to arrange themselves into groups similar to the adult condition.

At the age of flying—about fourteen or fifteen days after hatching—the crest is almost as densely pigmented as in the adult. The other portions, however, lack the density and grouped arrangement of the adult. I am unable to state the age at which complete pigmentation occurs, but it is some time after the bird is flying and has been using its eyes.

Pigmentation seems to be hastened and possibly started by the light stimulus. It begins in the sparrow's pecten at about the same time that the eye is exposed to the light, and in that part of the pecten most exposed to the light. This is also true of the chorioid in which pigmentation occurs first in the region exposed to the light and later in the posterior portions.

If light does act as a stimulus to the formation of pigment, the relative delay in the development of it in the pecten of the sparrow as compared to that of the chick may possibly be accounted for by the difference in the character of the nests of the two birds. The sparrow's nest is a very compact and bulky affair, often hidden in some recess or corner where the eggs and nestlings are in semidarkness. The hen's nest, on the contrary, is generally more exposed to the light and the hen's habit of leaving the nest for a time during the warmest and brightest part of each day would make the effect of light more pronounced. A comparison with some other closely related bird with an open nest would be of special interest in this regard. The fact that in the chick the eye functions within a few hours after hatching, while in the sparrow the eyes do not open until several days after hatching and they are then not exposed to bright light until about the time the bird leaves the nest, may also be a cause for the delay of pigmentation in the sparrow eye and again points to light as an influencing factor.

#### THE RETINA

As previously described under the early formation of the eye, the retina of the sparrow follows the same general order of development as in other vertebrates. The retinal, or nervous portion, is derived from the inner layer of the optic cup and the pigment epithelium from the outer layer.

With two days' incubation these two layers show little or no differentiation. They extend forward until they touch the lens. Where they come in contact with the lens they seem to have exerted sufficient force or resistance to growth to cause a bending in of its surface. This has exerted an influence also on the retina in that it tends to bend at the extreme margin. This is shown in figures 31, 48, and 49. The combined thickness at the anterior margin is 0.068 mm. and at the axial center 0.090 mm.

The pigment layer is one cell deep and devoid of pigment. It is thicker at the anterior margin (0.024 mm.) than at the axial center of the eye (0.016 mm.). At the anterior margin it bends inward and merges quickly into the retinal layer.

The retinal layer, according to Low ('08), is many cells thick. He bases his conclusion on the fact that the nuclei of this layer are scattered uniformly throughout its entire thickness. The cells are somewhat elongated and arranged with their long axes in a fairly definite radial manner. On the other hand, Keibel and Mall ('12), basing their conclusion on the position of the mitotic figures, claim that these cells form a single layer and that the nuclei alone are arranged in several layers. At the anterior margin this layer measures 0.044 mm. and at the axial center, 0.074 mm.

In the three-day embryo little change is noted. The retina is still in contact with the lens and shows the effect of pressure as previously described (fig. 32); the pigment layer is without pigment granules, and the nuclei of the retinal cells are still distributed throughout the layer (figs. 50 and 51). A slight difference is found in the thickness of these layers. The pigment epithelium measures the same at the anterior margin as in the two-day embryo, but is slightly thinner in the axial region where it measures 0.012 mm. The retinal layer is somewhat thicker, measuring 0.048 mm. at the lens and 0.076 mm. at the axial center.

Figure 52 shows the condition at four days' incubation. The margin of the retina is distinctly thinned as compared to the central region. It is still in contact with the lens, but does not form a depression in its surface as in the earlier ages. Though

the retina is relatively thinner at the anterior margin, it measures more than at the three-day age. The pigment portion at the lens measures 0.030 mm., the retina 0.056 mm., making a total of 0.086 mm. At the axis of the eye these layers measure, respectively, 0.009 mm. and 0.079 mm., making a total of 0.088 mm.

The pigment cells have become elongated in the region of the lens and have a few pigment granules in their inner ends. This occurs only on the temporal side of the lens. These granules are most abundant in the cells at the margin and grow less and less toward the optical axis until they wholly disappear, slightly posterior to the equator of the eye. This corresponds to the condition of pigmentation found in the 7-to-9-mm. human embryo, as described by Keibel and Mall. They say it develops first in the pupillary border and progresses backward to the optic stalk. They make no mention of pigmentation occurring in one border before the other. The fact that the pigment appears first in the region nearest the light indicates that, if not a cause of its formation, it is at least a stimulus to hasten its development.

The cells of the retina are beginning to migrate toward the inner surface of the layer. This leaves a space in the outer portion of the retina more or less free from nuclei. This space varies in width; it is widest at the lens, where it measures 0.006 mm. From this region it diminishes gradually in width toward the central part of the retina where it measures 0.004 mm. At this age a few ganglion cells have partially differentiated in the axial region. All of the other cells are elongated and spindle-like in form and have the radial arrangement previously described.

The fifth day of incubation shows some interesting changes. The combined thickness of the retinal and pigment portions has increased. At the lens the total thickness is 0.084 mm. and at the axial center it measures 0.116 mm. The dimensions of the retinal and pigment portions of these two regions at different ages of development are given in tables 4 and 5.

All of the cells of the pigment layer now show pigment granules. In the cells near the lens the granules are situated at the inner ends only. Slightly anterior to the equator of the eye

the granules are distributed rather uniformly throughout the cells. From this region back over the main portion of the retina the granules are either at the outer ends or along the sides of the pigment cells. Pigmentation is more dense and conspicuous in the cells near the lens than in the other regions of this layer.

The retinal layer shows a further differentiation of its cells in regard to location and form. A number of large, well-stained nuclei in the axial region, close to the pigment layer, are arranged in a row one cell deep. The cells are not always continuous, as they often have wide gaps intervening between them. This row of cells can be traced well forward, almost to the equator of the eye, where it blends with the main mass of nuclei. This is evidently the beginning of the outer nuclear layer which will later form the rods and cones (Kölliker, '83; Babuchin, '63; Max Schu'tze, '66; W. Müller, '74).

In the axial region another row of well-stained nuclei lies at the inner edge of the retina. This row is one cell deep and shows frequent wide spaces between the cells. A slight indication of nerve fibers is seen in this region. Toward the periphery of the retina this row of cells becomes less and less conspicuous until it disappears by blending with the main mass. This is the early differentiation of the ganglion cell layer. These two layers of cells are being formed by cells migrating from the main mass of cells.

Separating these two single-celled layers from the main mass of nuclei are two indefinite band-like regions with few nuclei. These are destined to become the inner and outer molecular layers. These two layers become indistinct and disappear toward the equator as the nuclear layers blend with the main mass of cells. At all points they show cells migrating inward and outward to form the ganglion cell and outer nuclear layers. The main mass of cells from which the above cells have migrated will become the inner nuclear layer.

Although this early differentiation can be made out by careful manipulation of the microscope, it is still too indistinct to show in the microphotographs (figs. 53 and 54).



In describing the differentiation of the retinal cells in the cat, Ramon y Cajal ('96) says that the multipolar cells (ganglion cells) are first to develop and are very soon followed by the growth of axis-cylinder processes (nerve fibers), then by the dendrites. He also says that at an early date the bipolar cells, which later form the rods, can be distinguished from those which will form the cones. Each sends processes toward the external limiting membrane and, later, one in the opposite direction. After a time the cone nuclei move to the external limiting membrane. He used the silver-nitrate method. In my sections I am unable to recognize at this age the two classes of bipolar cells.

Chievitz ('87) says that, with the exception of the nerve-fiber layer, the retina of the eighth-week human embryo is wholly epithelial in character. At this age two layers of nuclei are seen. An intermediate granular layer appears in the macular region in the five months' fetus. This latter age corresponds well with the development of the five-day sparrow.

In the seven-day sparrow embryo the pigment of the pigment epithelium is conspicuous and more dense than in the last age. The granules are situated at the inner ends of the cells at the lens region, but in the cells of the posterior part of the retina they are diffuse and no longer located at the sides or outer portions.

The first appearance of the pecten is seen at this age. In cross-section it is a conical projection of undifferentiated mesodermal cells from the optic disc. A detailed description of the development of this structure is given in the discussion of the pecten.

The retina shows further advance in differentiation. The various layers already described have become more noticeable and extend almost over the whole of the retina. They, however, still lack completion, being very indistinct. Between the outer row of cells (the outer nuclear layer) and the pigment layer in the axial region there now exists a narrow band-like region free from nuclei. These are the dendritic processes of these cells and represent an early stage in the formation of the rods and cones. In the human fetus, according to Chievitz, the cones begin to appear in the macular region at the seventeenth week.

The different layers of the retina are still too indefinite for accurate measurement. Tables 4 and 5 show that the total thickness of the retina in the axial region has increased to 0.138 mm., but at the lens it measures the same as the former age. This is readily observed in figure 59, which represents a section through the center of the eye of a seven-and-one-fourth-day embryo. Some of the differentiation into layers above described can be seen. The anterior margin of the retina is still in contact with the lens and a differentiation into pars optica and pars caeca has not yet occurred.

At the eight day of incubation the thickness of the retina has markedly increased in the axial region (fig. 64). This is mainly due to an increase in the retinal portion. The different retinal layers, with the exception of the inner and outer molecular layers, have now become sufficiently distinct to measure. These measurements are given in table 4. The dimensions given for the inner nuclear layer includes that of the two molecular layers. At this age a space 0.016 mm. wide, and free from nuclei, is situated between the single row of ganglion cells and the inner margin of the retina. This is the nerve-fiber layer, but no nerve fibers were visible in the sections used.

A delicate line, consisting mainly of very small, brightly stained bodies arranged in a row, runs parallel with the outer margin and about midway of the outer clear zone (rod-and-cone region). They appear to be situated on radial projections passing through the outer nuclear layer. Some of these projections can be traced to cells in the middle nuclear mass. This delicate line is the external limiting membrane and the radial projections are the supporting fibers (Müller's fibers). According to Ramon y Cajal, the supporting structures of the retina, which correspond to the glia cells of the central nervous system, develop at an early age. Their nuclei are at first scattered, but later are grouped in the inner granular layer.

Several blunt processes project from the cells of the outer nuclear layer as far as the limiting membrane and other shorter processes, of various lengths, are the forming rods and cones. This corresponds to the ten-day chick embryo as described by

Hertwig ('90). M. Schultze ('66) says that in rabbits and cats (born with their lids closed) the rods and cones are seen for the first time soon after birth. In man they are formed two months before birth (Keibel and Mall).

TABLE 4

*Showing the thickness at the axis of the different layers of the retina, the total thickness, the thickness of the choroid and sclerotic layers at different ages. Measurements are in millimeters, and as nearly as possible perpendicular to the retina in the axial region. 2 to 13 represent the ages in days of the embryos used; H-2, hatched two days; H-4, hatched four days; Fov. c, H-fl, and Area H-fl, center of fovea and area of young just flying; A-Fov, center adult fovea; A-a, thickness of area of adult 0.360 mm. from center of fovea*

AGE	NERVE FIBER LAYER	GANGLION CELLS	INNER MOLECULAR	INNER NUCLEAR	OUTER MOLECULAR	OUTER NUCLEAR	RODS AND CONES	PIGMENT EPITHELIUM	TOTAL RETINA AND PIGMENT	CHOROID	SCLERA	
											Cartilage	Fibrous
<i>days</i>												
2				0.074				0.016	0.090			
3				0.076				0.012	0.088			
4				0.079				0.009	0.088			
5				0.108				0.008	0.116			
7½				0.132				0.006	0.138			
8	0.016	0.004		0.144		0.004	0.004	0.007	0.179	0.008		0.024
10	0.020	0.008	0.012	0.080	0.006	0.006	0.114	0.004	0.150	0.012	0.012	0.004
12	0.016	0.026	0.022	0.096	0.012	0.012	0.004	0.008	0.196	0.015	0.016	0.004
13	0.020	0.024	0.028	0.116	0.012	0.014	0.008	0.004	0.226	0.016	0.012	0.008
H-2	0.028	0.028	0.024	0.096	0.008	0.012	0.004	0.006	0.206	0.012	0.012	0.012
H-4	0.016	0.024	0.032	0.068	0.010	0.010	0.008	0.008	0.176	0.016	0.012	0.012
Fov. H-fl	?	0.060	0.032	0.068	0.006	0.010	0.012	0.012	0.200	0.040	0.036	0.020
Area H-fl	?	0.040	0.052	0.116	0.006	0.008	0.012	0.014	0.248	0.040	0.040	0.020
A-Fov	?	0.032	0.008	0.034	0.010	0.020	0.044	0.032	0.180	0.152	0.044	0.004
A-a	0.016	0.028	0.060	0.132	0.012	0.024	0.020	0.032	0.328	0.116	0.042	0.004

Differentiation into layers has proceeded much more rapidly in the axial region than at the lens border. A gradual decrease is noticed from the central part of the retina toward the periphery. At the anterior margin it is impossible to distinguish these layers (table 5). The undifferentiated cell mass here fills the whole of the retinal layer. The margin of the retina at the lens is relatively thin compared with the axial region. It increases in thickness very slowly toward the macular region

for a short distance, then much more rapidly. This thinner region may be termed the first indication of the pars caeca. There is, however, no decided mark to indicate its extent. At this time changes are taking place in the adjacent pigment and chorioid layers, which is the early formation of the ciliary bodies.

The nine-day embryo shows a decided reduction in thickness of the retina a short distance from the pupillary border (figs.

TABLE 5

*Measurements in the region of the ora serrata of the different layers of the retina, the whole retina, the chorioid and sclerotic layers at different ages. Measurements are in millimeters, 2 to 13 represent the age in days of the embryos used; H-2, hatched two days; H-4, hatched four days; H-fl, young just flying*

AGE	NERVE FIBER LAYER	GANGLION CELLS	INNER MOLECULAR	INNER NUCLEAR	OUTER MOLECULAR	OUTER NUCLEAR	RODS AND CONES	PIGMENT EPITHELIUM	TOTAL RETINA AND PIGMENT	CHORIOID	SCLERA	
											Cartilage	Fibrous
<i>days</i>												
2				0.044				0.024	0.068			
3				0.048				0.024	0.072			
4				0.056				0.030	0.086			
5				0.056				0.028	0.084			
7½				0.060				0.024	0.084			
8				0.044				0.028	0.072			
10		0.024	?	0.096				0.004	0.024	0.016	0.016	0.044
12		0.016	?	0.140				0.008	0.164	0.008	0.016	0.028
13	0.008	0.024	0.020	0.088	0.008	0.012	0.004	0.008	0.172	0.004	0.016	0.020
H-2	0.012	0.026	0.020	0.074	0.008	0.010	0.008	0.006	0.164	0.024	0.032	0.020
H-4	0.010	0.014	0.032	0.052	0.012	0.012	0.006	0.007	0.145	0.032	0.012	0.024
H-fl	0.006	0.026	0.040	0.032	0.004	0.009	0.007	0.006	0.130	0.024	0.024	0.008
Adult	0.004	0.036	0.024	0.024	0.004	0.012	0.012	0.012	0.128	0.016	0.020	0.012

38 and 69). This rather abrupt thinning of the retina occurs 0.4896 mm. from its anterior margin. It is the first indication of the true ora serrata so far observed. The thin portion, the beginning of the pars caeca, extends to the lens, with which it is in close contact. This modification in thickness is more noticeable on the temporal than on the nasal side of the lens. Associated with this change in the retina is the noticeable advance in the development of the ciliary body and the iris. A portion of the retina and pigment layer extends over the front of the

lens, with the developing stroma of the iris. The pars caeca can now be distinguished as pars ciliaris and pars iridica. Keibel and Mall claim that the decrease in thickness of the pars caeca is due to the more rapid growth of the ciliary body and the ciliary processes. In other words, the retinal part does not grow so fast, resulting in a drawing out of its surface.

In the axial region the molecular layers are becoming more distinct. The cells in the middle layer are thinning out by migration to either side.

In the peripheral part of the retina little or no differentiation can be discerned. The embryonic cells still occupy the whole width of the retinal layer.

The pigment layer shows a marked reduction in thickness at the ora serrata and is now of almost uniform thickness throughout (tables 4 and 5).

In the axial region of the ten-day embryo the retina shows much advancement in differentiation. The nerve-fiber layer is wide and entirely free from nuclei. The ganglion-cell layer is thicker and composed of two rows of cells, which still retain their embryonic form, but nerve-fiber processes could not be demonstrated in the sections used. This layer is completely separated from the inner nuclear layer by the inner molecular, which is now free from nuclei in this region. Toward the periphery this double row of ganglionic cells gradually becomes a single row, then finally disappears by blending with the main nuclear mass, due to the disappearance of the inner molecular layer.

The outer nuclear layer is wider than in the nine-day chick, and is composed of two rows of closely packed cells. This layer is not so sharply marked off from the inner nuclear mass as the ganglion-cell layer, because of a number of cells scattered through the outer molecular layer.

The outer limiting membrane is about 0.004 mm. outside the outer nuclear layer. Beyond this is a light-colored layer 0.014 mm. wide which corresponds to the rod-and-cone layer. But the rods and cones are as yet lacking in this region. All of these layers become less and less distinct toward the periphery of the retina, and finally blend with the embryonic nuclear mass.

The pigment layer is reduced somewhat in thickness and the granules are confined within the cell bodies, showing no tendency to migrate.

The retina at the age of hatching, about the twelfth or thirteenth day of incubation, has greatly increased in thickness, both in the axial region and at the ora serrata (tables 4 and 5). The different layers have become more sharply defined and the differentiation has extended farther toward the periphery.

At this age is noticed for the first time the early beginning of the fovea centralis. This is not any special modification of the retina itself, but a thickening of the chorioid coat, caused by an increase in the blood-vessels in this region. A more complete discussion of the development of the fovea will be given under a separate heading.

The modification into the pars ciliaris retinae is more pronounced and lengthened. The pigment layer and a thin portion of the retinal layer have pushed farther forward over the front of the lens, forming the posterior part of the developing iris (figs. 41 and 42). This is described in detail in dealing with the development of the cornea, iris, and aqueous chamber. The ora serrata is now 0.748 mm. from the lens.

The ganglion-cell layer in the axial region is now two to three cells deep. Some processes extend from these cells into the nerve-fiber layer. These are the nerve fibers which also extend along the nerve-fiber layer. A few can also be demonstrated in the optic nerve, but the bulk of the nerve is still composed of numerous elongated, spindle-like cells, characteristic of the earlier ages.

Light, faintly stained, radial bodies occur in the rod-and-cone layer. They are not closely arranged and are of various lengths. Many of these developing rods and cones reach almost to the pigment layer. There is no migration of the pigment granules toward these processes.

Two days after hatching the retinal layers are rather sharply defined throughout the whole extent of the retina (figs. 82, 83, and 84). In the axial region there is a general increase in the total thickness of the retina. The layers which show a notice-

able thickening are the nerve fiber, the ganglion cell, and the inner molecular (table 4). At the ora serrata the retina has begun to thin, largely due to a reduction in thickness of the inner nuclear layer (table 5). The ganglion-cell layer is three and occasionally four cells deep. This is true throughout its extent. A noticeable difference is observed in the arrangement of these cells in different parts of the retina. In the axial center they are very compact, but in the periphery they are loosely arranged, though still conforming to a row three cells deep. The inner nuclear layer is about three cells deep, and the cells show about the same general arrangement throughout its extent. The five divisions of the inner molecular layer described by Ramon y Cajal can be distinguished.

The pigment layer is now very densely pigmented, but the granules are wholly within the cell bodies. Tangential sections through these cells show that the granules are located largely in the peripheral portions of the cell, leaving the nuclear region almost free.

A continued decrease in the total thickness of the retina is observed in the young sparrow four days after hatching. This is due, in my opinion, to a spreading of the retinal cells over a wider area to keep pace with the rapid increase in the size of the eye. The great enlargement in the surface of the retina is shown in table 6. So far as I have been able to observe, there is no multiplication of the cells of the retina after hatching. There is, however, an increase in the size of some of them, especially the ganglion cells. Evidence indicates that extension of the retina in a peripheral direction, due to growth of the eye, is accomplished by a migration of the retinal cells in this direction. The reduction of the ganglion cells to a layer one cell deep, and a lack of continuity of these cells, as well as a reduction in number of cells of the other nuclear layers, point conclusively to a migration unaccompanied by an increase in the number of cells.

At this age the pigment cells show that a very slight movement of pigment granules toward the rod-and-cone layer has occurred.

The inner segments of the rod-and-cone processes are broad and blunt. Their nuclei occupy their inner ends. At the outer ends of these blunt processes are very short processes, the beginning of the outer segments. No distinction can be made in these elements in my sections as to whether they are rods or cones.

TABLE 6

*Showing the total thickness of the retina at the ora serrata and the axial regions, the computed area of the total retina, and the axial and equatorial diameters of the eye at the ages indicated. 2 to 12, number of days incubation; H-2, hatched two days; H-4, hatched four days; H-fl, young just flying. All measurements are in millimeters*

AGE	TOTAL THICKNESS OF RETINA		COMPUTED AREA OF RETINA	DIAMETER OF EYE	
	Ora serrata	Axial region		Axial	Equatorial
<i>days</i>	<i>mm.</i>	<i>mm.</i>	<i>sq. mm.</i>	<i>mm.</i>	<i>mm.</i>
2	0.068	0.090	1.124	0.629	0.833
3	0.072	0.088	2.340	0.748	1.200
4	0.086	0.088	3.051	0.833	1.377
5	0.084	0.116	5.651	1.360	1.870
7	0.084	0.138	6.980	1.495	2.080
8	0.072	0.179	12.610	2.015	2.795
9	0.130	0.160	16.370	2.405	3.185
12	0.164	0.196	30.410	3.712	4.032
H-2	0.164	0.203	37.440	4.224	4.800
H-4	0.145	0.176	57.430	5.440	5.952
H-fl	0.130	0.248	77.410	5.888	6.912
Adult	0.128	0.324	85.000	6.192	7.236

The ganglion cells have developed dendritic processes and appear to have reached the complete differentiation seen in the adult.

The five divisions of the inner molecular layer described by Ramon y Cajal are more conspicuous.

The amakrin cells of the inner nuclear layer are separated from the bipolar cells by a narrow lighter region.

Five to six days after hatching, all of the layers of the retina have practically reached the condition of the adult, excepting the rod-and-cone layer. The outer segments of these elements have increased in length, but have not reached the adult con-



dition. The inner segments are also thicker than in the adult and are in direct contact with their nuclear portions. In no case are the long, slender portions between the nuclei and the cones, common to the adult, observed. The oil droplet common to the adult cones is not visible. Distinctions between rods and cones cannot be made in my sections. The fact that the rods and cones are almost the last elements of the retina to develop has been demonstrated by Hertwig and other investigators in other animals.

The migration of the pigment granules is becoming more conspicuous. This migration appears as blunt, rounded projections which are so dense as to resemble a black mass. In the six-day young these rounded pigment projections begin to fray out at their inner ends so that some individual streams of granules can be observed.

About this time the eyes of the sparrow begin to open. The most conspicuous change in the developing retina is the migration of the pigment granules. This is no doubt due to the stronger light stimulus. The external segments of the rods and cones are so masked by these granules that they are scarcely visible. Very few rods are seen. The inner segments of the cones are 0.004 mm. in diameter. Numerous twin cones are seen at this age. In a tangential section the inner segments of these twin cones measure 0.004 by 0.006 mm. in diameter.

As development continues, the cones gradually recede from their nuclei, so that these structures are connected by the elongated portion of the cell characteristic of the adult.

The ganglion-cell layer shows the gradual thinning toward the periphery until the adult condition is reached. That is, it consists of a single row of cells which are relatively widely separated from each other.

The outer nuclear layer and the rod-and-cone elements become reduced toward the periphery until the cones are relatively few and not closely arranged. The cones are also shorter and thicker at the periphery than at the optical axis.

Though the retinal layers are differentiated into practically the adult condition at a relatively early age, the full size of the

eye and the consequent total area of the retina do not reach the adult condition until some time after the bird has left the nest and is able to fly. Table 6 shows that the total area of the retinal expanse at the age of flying is 77.410 sq. mm. This is almost 8 sq. mm. less than that of the adult. The dimensions of the eye in the axial and equatorial directions are correspondingly less than that of the adult.

The blood supply to the retina in birds differs from that in mammals. The outer layers in each case are nourished by osmosis from the vessels of the chorioid. The inner layers receive their nourishment in a different manner. Early in the development of the eye in mammals, the retinal artery makes its appearance. This artery is distributed over the inner surface, supplying all the layers of the retina, except the outer nuclear and rod-and-cone layers. In the bird there is no retinal artery nor do blood-vessels directly supply nourishment to the retina as in mammals. The statement of O. Hertwig that lampreys do not have blood-vessels in their retina, but that all other vertebrates do, is not true. In birds the inner layers of the retina are nourished by osmosis from the blood-vessels of the pecten. The development of the blood supply to the eye, therefore, corresponds to the development of the pecten and the chorioid.

#### THE FOVEA

The development of the fovea has been studied by a number of investigators. Chievitz ('87), who has made many observations on the embryonic development of the eyes of birds and other vertebrates, states that the fovea begins as a thickening of the retina. This thickened region he calls the area centralis. In the center of this, area differentiation of the retinal cells into layers is first observed. This region is later thinned out, or pitted in by a radial migration of the cells from the center to the sides. Numerous other investigators have verified these facts.

In my study of the development of the fovea of the sparrow I agree in general with the results of Chievitz, but draw different

conclusions. I have found that the retina is thicker at the optical axis than elsewhere, and that differentiation into layers begins at that point. This thickening and differentiation is gradual and general, and hardly characteristic of the true area centralis which occupies a rather definite region, which is first indicated by a special modification of the chorioid at the optical axis.

The first indication of the developing area and fovea centralis which I have found in the sparrow antecedes that described by Chievitz. It is not a local thickening of the retina, but a special thickening of the chorioid coat, immediately back of the region where the fovea later develops. This is not at first accompanied by any observable change in the retina. The special thickening of the chorioid is entirely distinct and easily distinguishable from the general increase in thickness of the chorioid in the axial region as compared with the region of the ora serrata.

As early as the tenth day of incubation the chorioid coat shows a marked increase in thickness from the periphery to the axial region where it is thickest. This, however, is a gradual augmentation and not the pronounced and special increase which determines the location and formation of the fovea. It is nevertheless true that the region of maximum thickness is where the fovea will develop. As shown in figure 70, there is no special modification of the retina in this region at this age. The retina, however, has its greatest measurement at this point.

At twelve to thirteen days' incubation, the age of hatching, the chorioid measures about 0.008 mm. at the ora serrata and gradually increases to 0.015 mm., in the central portion. In a small region at the optical axis it rapidly increases to a maximum thickness of 0.0200 mm. This increase, due to a greater number of blood-vessels, marks the location of the area centralis and later the fovea.

Closely following this richer blood supply is a rapid increase in the number of retinal cells accompanied by a marked thickening of the chorioid (fig. 86). By the fourth day after hatching the condition of thickened retina is rapidly followed by a slight thinning of the retinal cells in the optical axis due to a radial migration from this point (fig. 91).

Chievitz ('87) says that in the human fetus the fovea begins to form after the sixth month and is completed at seven and one-half or eight months. In this process he finds that there is a thinning of the ganglion-cell layer from seven cells deep to a single row of cells in the center of the adult fovea. He further says that all the retinal layers reach the adult condition two and one-half months before birth. If he means by this that the fovea is completely formed at this age, there is a discrepancy in his statements.

Five or six days after hatching the special region of the chorioid shows a great mass of blood-vessels (figs. 95, 96, 97, and 103). The center of the fovea is shown in figure 95. All the layers of the retina are present, but they show a slight decrease in thickness in the immediate center.

By the tenth day the pitting has become more pronounced and the thickness of the chorioid much augmented. The inner nuclear layer and the molecular layers show the greatest thinning.

From this age on to the adult condition, as the fovea becomes more perfect, the special thickening of the chorioid is reduced and practically disappears with the complete formation of the fovea. The chorioid, however, like the different layers of the retina, is much thicker in the region of the optic axis than at the ora serrata. The maximum thickness at the fovea is 0.134 mm. and the minimum at the ora serrata is 0.016 mm. Also, the reduction in thickness is gradual, and the special area at the earlier ages immediately back of the fovea has entirely disappeared. On the temporal side of the fovea the chorioid maintains almost a maximum thickness for about half the distance to the ora serrata. This is not true of the nasal side. The physiological reason for this, in my opinion, is the fact that this portion of the retina is used more by the bird than the part on the nasal side of the fovea. Owing to the lateral position of the eyes in the head, the rays of light from the fields of vision in front of it would necessarily stimulate the temporal portion of the retina more than the nasal side. Since the existence of the bird depends more on perception of the visual fields in front of it and in the line of the optical axis than in any other direction,

these portions of the retina will expend a greater amount of energy. A richer blood supply is therefore necessary. This adaptation also indicates that it is very probable that the sparrow has binocular vision with the temporal regions of the retina. It would require but a slight convergence of the eyes to accomplish this. Experiments made with sparrows with one eye extirpated, prove that the bird can easily move its eyes sufficiently to bring about binocular vision to within 1 mm. of the fovea. Many birds have a second fovea located in this temporal region which is used in binocular vision (Slonaker, '97 and '18).

The fact that the area and fovea centralis do not begin to show development in the sparrow until about the age of hatching, and that the most rapid differentiation occurs after the eyes open, indicates that the light stimulus may exert a great influence in its formation. In this connection it is interesting to note that Held ('96) has found in animals born with their eyes closed the stimulus of light hastens the development of myelination in the optic-nerve fibers.

#### THE MUSCLES OF THE EYE

*Extrinsic muscles.* The development of the extrinsic muscles begins at a much earlier age than that of the intrinsic muscles. At the age of three days' incubation some of the mesoderm, in regions at the posterior part of the orbit, begins to group itself into very indistinct masses of more closely packed cells. They are more condensed near the optic-nerve entrance and grow less dense and distinct as they are traced toward the eyeball.

By the fifth day these masses are more sharply marked off from the rather loosely arranged surrounding cells. The cells composing these masses have become slightly spindle-shaped in outline. They are arranged with their long axes parallel to the greatest dimension of the mass and in general parallel to the surface of the eye. These changes continue rapidly, so that by the seventh day the elongation of these cells and their parallel arrangement is easily demonstrated (fig. 56, *M*). The cells at the distal ends of these masses gradually blend with the cells forming the sclerotic coat.

By the ninth day of incubation the extrinsic muscles have become much more sharply defined, both as to shape and connections (figs. 69 to 72, *M*). These cells, though they show great elongation and definite parallel arrangement, still lack the differentiation of striated muscle. At this age the wall of the orbit is cartilaginous, very closely resembling the cartilage cells of the sclerotic coat.

Although these formative muscle masses increase in extent to keep pace with the rapidly increasing size of the eyeball, there is no appearance of striation in the muscle until two days after hatching. At this age very faint and indistinct cross striations are barely visible, but by the fourth day after hatching they are more easily demonstrated, but are still far from complete. The muscles of the upper and lower lids, the suprapalpebral and infrapalpebral, show occasional faint striations, most pronounced in the infrapalpebral near its proximal attachment to the lid.

About the sixth day after hatching all the extrinsic muscles are clearly striated. The significance of this is easily understood when we consider the fact that the eyes of the nestling open about this stage. Previous to this time there was no occasion for physiological activity of these muscles. It is also interesting to note that the infrapalpebral muscle, which is the principal one involved in the act of opening the eye of the bird, becomes striated earlier than the suprapalpebral muscle.

*Intrinsic muscles.* The intrinsic muscles of the eye—the ciliary muscles and the muscles of the iris—appear at a much later stage than the extrinsic. Although the formation of the iris has fairly well advanced by the twelfth day of incubation and the ciliary bodies are beginning to show, no indication of muscle tissue can be observed. The cells of these structures still lack differentiation.

At the age of hatching a few spindle-like cells appear between the ciliary bodies and the sclerotic coat. They are fairly definitely arranged and seem to have been derived from the same cells as those which form the sclerotic coat. These later develop into the ciliary muscles.

A single row of similar cells occurs in the posterior portion of the iris, between the pigment portion and the mass of tissue in front of it. This row later forms the radial muscles of the iris. The cells which lie in front, and which will later form the circular, or sphincter muscles of the iris, appear as rounded nuclear bodies. This is due to the plane of section cutting these cells at right angles to their long axes. The different views as to the origin of these muscles are discussed in dealing with the development of the cornea, iris, and aqueous chamber.

Two days after hatching these structures have become more typical of muscle tissue. The cells have become greatly elongated, the mass more definite in shape, and a few faint striations can be made out in the muscle cells. By the fourth day these cross-striations have become more pronounced and can be easily observed. Development proceeds rapidly, so that by the time the eyes open these muscles are fully striated and ready to function.

#### THE EYELIDS

The first appearance of any of the structures of the lids is seen in the chick at the age of about sixty-four hours' incubation, just after the complete formation of the lens vesicle. The ectoderm, now covering the lens, becomes later the epithelium of the conjunctiva (figs. 45, 46, and 47). At this age the ectoderm covering the head and eyes shows no differentiation. In the chick, even at the age of 100 hours, this layer is still uniform over the surface.

The earliest age of the sparrow (about two days) which I have secured corresponds fairly closely to the four-and-one-half-day chick, and is slightly further advanced than that of the 100-hour chick shown in figure 47. The sparrow at the age of two days is shown in figures 1, 17, and 48.

The first indication of the lids is seen in the four-day sparrow embryo (figs. 20 and 54, *N*). In the five-day sparrow embryo it is more pronounced. It consists of a slight swelling on the surface at the anterior margin of the eyeball, which is due to an increase, or outgrowth, of mesodermal cells and not to any

change in the ectoderm. This is the beginning of the nictitating membrane, or third eyelid. This elevation increases in size, and at the same time begins to grow backward over the eye. At the sixth day a second swelling appears just anterior to that of the nictitating membrane (fig. 21), and a similar thickening occurs at the opposite side of the eye. These swellings are the beginnings of the true lids. Up to this age the full size of the eyeball is very conspicuous, as it is covered only by the thin ectoderm. By the seventh day these elevations have become quite prominent, and those forming the upper and lower lids have extended completely around the eye (figs. 22 and 56). They seem to be growing out over the eye, but this appearance is due more to the increasing size of the eyeball than to an encroachment of the forming lids.

This rapid growth of the lids and the eyeball continues, so that by the ninth day the nictitating membrane has grown backward almost to its full extent (fig. 26 and 69). The ectoderm, which covers the cornea, completely encloses the nictitating membrane, and lines the ocular side of the lids, has become more epithelial-like in character. At the extreme anterior fornix, where it is reflected from the cornea over the nictitating membrane and finally from the anterior surface of this membrane to the lining of the lids, it presents a uniform and smooth surface. At the free margin of the lids the ectoderm is beginning to thicken slightly.

At this age the first slight elevations of the plumules of the lids and feathers of the head begin to appear. They are first developed on the upper part of the head and the upper lid. The interpalpebral space is still quite large. The ratio of the diameter of this space to that of the eyeball is 9:11.

The nictitating membrane has become somewhat thinner, but in cross-section is still decidedly club-shaped, bending slightly to conform to the surface of the cornea. The thickest portion is toward the free margin and the thinnest at the attachment at the fornix. The thickness at these two regions is 0.112 mm. and 0.040 mm., respectively. The cells constituting the central portion of the nictitating membrane are widely separated, as compared with the corresponding portion of the true lids.



The appearance suggests that the increase in the number of mesodermal cells has not kept pace with the growth in size of this structure and that the latter is due to an inflow or secretion, of a clear intercellular fluid. Blood-vessels extend almost to the free margin.

At twelve days' incubation (fig. 75) the nictitating membrane is beginning to differentiate into the marginal plate and a much thinner proximal portion, somewhat like the adult. The conjunctival epithelium is thicker in the axial part of the cornea than elsewhere. Here it is composed almost wholly of columnar cells with about one thin layer of the stratified type at the outer margin. From this region it diminishes gradually, from 0.024 mm. in thickness, to the fornix, where it measures 0.012 mm. It maintains about this thickness over the nictitating membrane and the ocular surface of the lids. Throughout its entire extent it forms a smooth covering on these surfaces. At a later period of development the conjunctiva is thrown into numerous folds at the fornix conjunctivae. This arrangement allows free movement of the lids and eyeball without injury to the epithelium.

By the thirteenth day, when the bird hatches, all of the lids have reached practically the adult structure and form (fig. 30). The nictitating membrane has become thin and the marginal plate is well developed and stiffened by a cartilage-like plate. This doubtless corresponds to the plate of hyaline cartilage which Schäfer ('97) says is found in the nictitating membrane of many adult animals. The marginal folds of the lids are conspicuous. The plumule papillae are well developed, but the plumules do not appear until some time after hatching. The ratio of the horizontal diameter of the interpalpebral space to that of the eye is as 13:40. This is practically the same as that of the adult, which is 1:2. The ratio in the vertical direction cannot be estimated, as the lids are partly closed.

At two days after hatching, folding of the epithelium at the fornix conjunctivae has begun. This is shown in figures 97, 98, 99, 100, and 102. The greatest folding of the conjunctiva occurs on the base of the nictitating membrane. The structure of the lids is now practically the same as has been described for the adult (Slonaker, '18).

## THE LACRIMAL GLANDS

The lacrimal secretion in the bird is supplied by two glands, the lacrimal gland and Harder's gland, each of which is connected with the conjunctival sac by a single duct (Sardemann, '87). The lacrimal gland, which is smaller, is located in the orbit slightly below and temporal to the posterior canthus. Its secretion is directed onto the temporal portion of the lower lid. Harder's gland is a large, tubular gland (MacLeod, '80), located back of the eyeball and covers about one-third of its posterior surface. Its duct opens into the bottom of the conjunctival sac formed by the nictitating membrane and the eyeball. Since the conjunctival surface in the bird is kept moist and clean principally by the nictitating membrane, this portion of the eye would naturally need the greatest amount of lacrimal secretion which accounts for the large size of the harderian gland.

The lacrimal glands are developed from the conjunctival epithelium (Keibel and Elze, '08; Speciale-Cirincione, '08; Hertwig, '90). They first consist of buds of epithelial cells from the conjunctival sac which grow backward from the regions which later mark the outlets of the ducts from these glands. In the bird a single bud, which is destined to form the lacrimal gland lies at the temporal canthus; that which will form the harderian gland arises from the pocket under the nictitating membrane. In man the lacrimal gland is formed by a number of buds or solid projections of epithelial cells which start about the seventieth day of development. In the sparrow a single bud is formed and grows backward as a solid, cylindrical mass of cells just outside of, and parallel to, the surface of the eyeball. When it reaches the normal location of the gland it branches into a number of lobes. The first appearance of this gland was seen in the sparrow about the eighth day of incubation. This backward growth is rapid. By the ninth day, lobes of the lacrimal gland are observed. They consist of fairly definite arrangement of cells which, in cross-section, form almost a circle. The cells forming the outer wall are of uniform size and enclose other cells to make a compact mass. The location

of the lacrimal gland is indicated by a group of blood-vessels long before the gland cells appear. Blood-vessels also run parallel to the path of the developing gland and duct. In fact, the direction of growth is indicated by a richer blood supply. The growing bud forms a more or less cylindrical bar of almost uniform diameter which runs in a tortuous manner from the conjunctiva to the region of the gland. Here it branches and forms the four or five lobes of the gland. The gland increases in size until the fifth or sixth day after hatching, when it has reached almost the adult condition except in size. During this time there has been a disintegration of the cells in the center of the lobes of the gland and in the connection to the conjunctiva which results in the formation of the lumen. It is now ready to function.

According to Kirchstein ('94), the lacrimal gland has not reached its full development in the newborn child, being only one-fourth to one-third as large as the adult gland. This has been confirmed by Goetz ('08). There is also a difference in the appearance of the cells (Axenfeld, '99). It is claimed by De Wecker ('99) and Baratz ('02) that, even though the lacrimal gland is not completely developed at birth, it is nevertheless capable of functioning. This condition corresponds favorably to the development in the sparrow a few days after hatching, when the eyes open.

The development of the harderian gland is very similar to that of the lacrimal. It makes its appearance at a slightly earlier date. At seven and three-fourths days' incubation a solid cylindrical bar of epithelial cells extends backward from the conjunctival pouch, formed by the nictitating membrane and the eyeball, to a short distance beyond the equator of the eye. Its course is more direct and less tortuous than that of the lacrimal gland. The diameter gradually increases with the backward growth. This growth continues until, by the thirteenth day, it has reached almost as far as in the adult. It has increased greatly in size, but as yet shows no lumen. The size increases until, by the second day after hatching, it covers a relatively large part of the proximal surface of the eyeball.

While this is taking place the disintegration of the central cells of the gland and duct has occurred, similar to that in the lacrimal gland. The gland and duct now have a lumen. Five or six days after hatching the gland is ready to function. This is about the time the eyes open.

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## PLATES

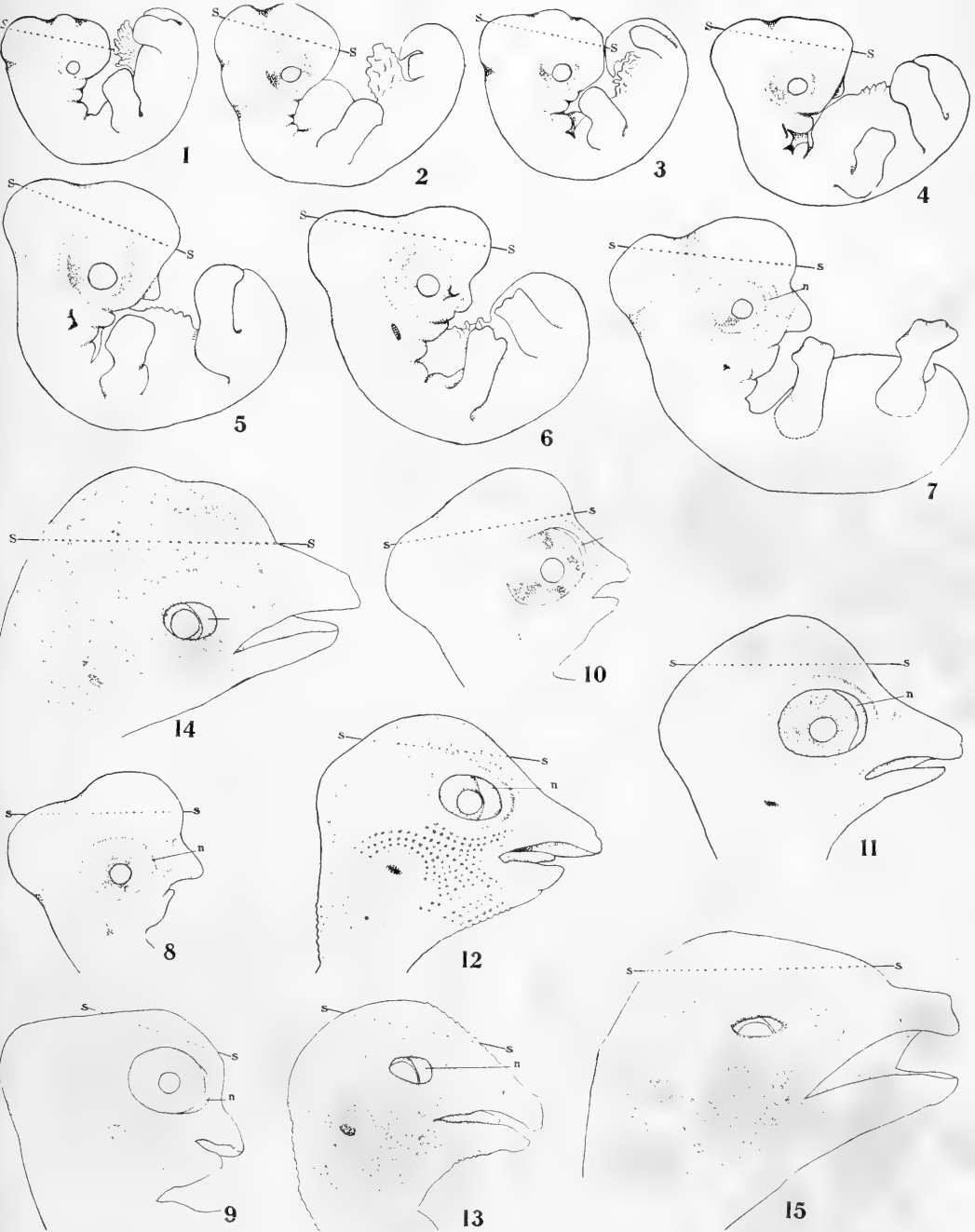
## PLATE 1

### EXPLANATION OF FIGURES

Enlarged outline camera-lucida drawings of different ages of the English sparrow, showing the development of the external features of the eye. *S-S*, represents the plane of section for microscopic study represented in succeeding microphotographs; *n*, nictitating membrane.

- 1 Embryo 2 days old.
- 2 Embryo  $2\frac{1}{2}$  days old.
- 3 Embryo 3 days old.
- 4 Embryo 4 days old.
- 5 Embryo 5 days old.
- 6 Embryo 6 days old.
- 7 Embryo 7 days old.
- 8 Embryo  $7\frac{1}{4}$  days old.
- 9 Embryo  $7\frac{3}{4}$  days old.
- 10 Embryo 8 days old.
- 11 Embryo 9 days old.
- 12 Embryo 10 days old.
- 13 Embryo 11 days old.
- 14 Embryo 12 days old.
- 15 Embryo 13 days old.



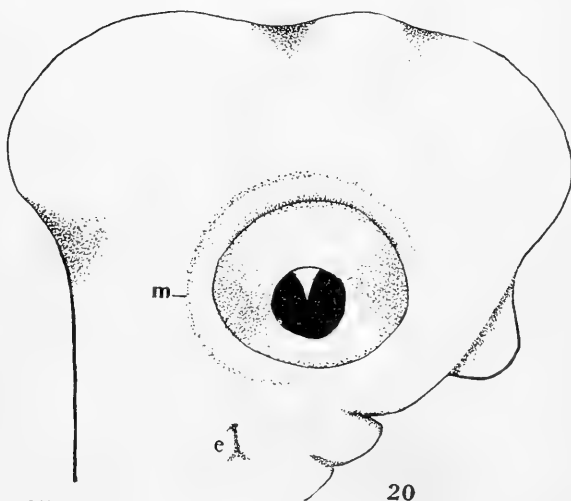
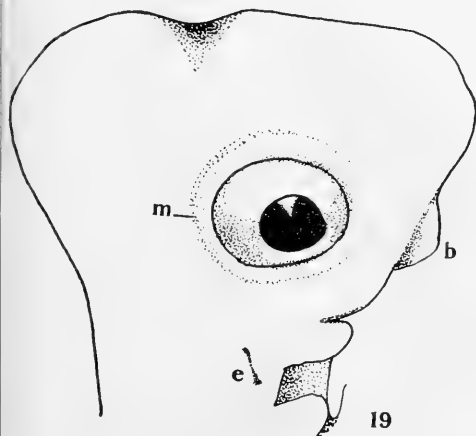
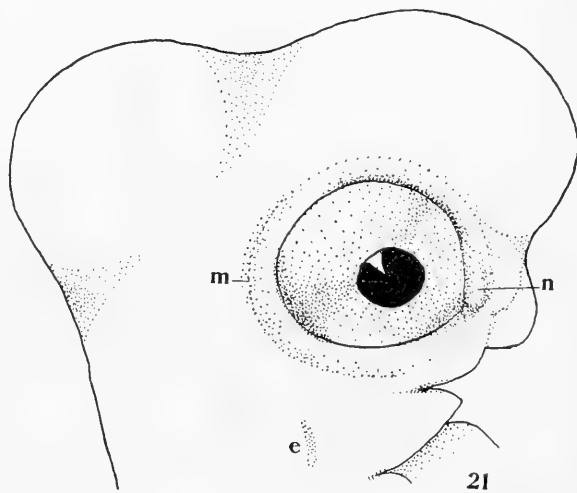
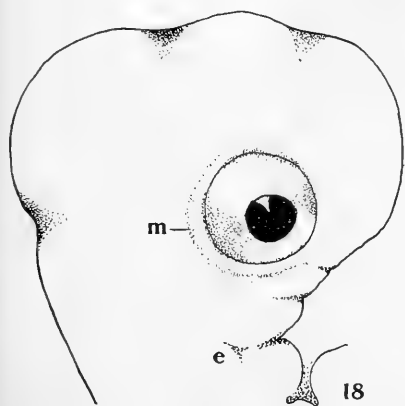
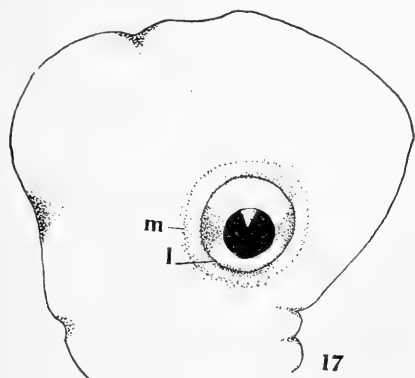


## PLATE 2

### EXPLANATION OF FIGURES

Very much enlarged camera-lucida drawings, showing the development of the external parts of the English sparrow eye. *b*, beginning of beak; *c*, chorioid fissure; *e*, ear pit; *l*, margin of lids; *m*, outline of eye as seen through the ectoderm; *n*, beginning of the nictitating membrane.  $\times 15$ .

- 16 Head of embryo 2 days old.
- 17 Head of embryo  $2\frac{1}{2}$  days old.
- 18 Head of embryo 3 days old.
- 19 Head of embryo 4 days old.
- 20 Head of embryo 5 days old.
- 21 Head of embryo 6 days old.



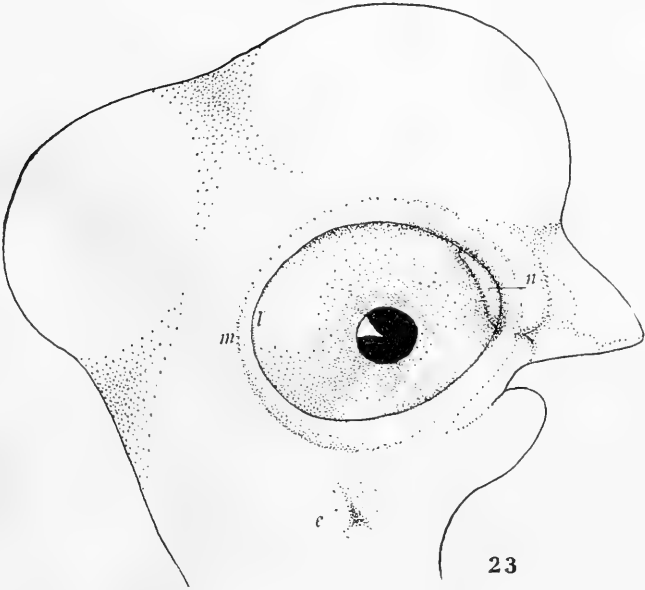
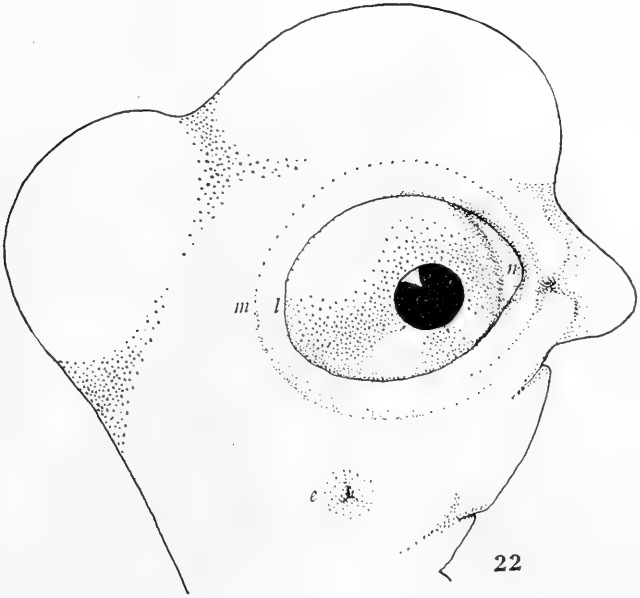
### PLATE 3

#### EXPLANATION OF FIGURES

Very much enlarged camera-lucida drawings, showing the development of the external parts of the English sparrow eye. *e*, ear pit; *l*, edge of lids; *m*, outline of eye showing through the ectoderm; *n*, nictitating membrane.  $\times 15$ .

22 Head of embryo 7 days old.

23 Head of embryo  $7\frac{1}{4}$  days old.

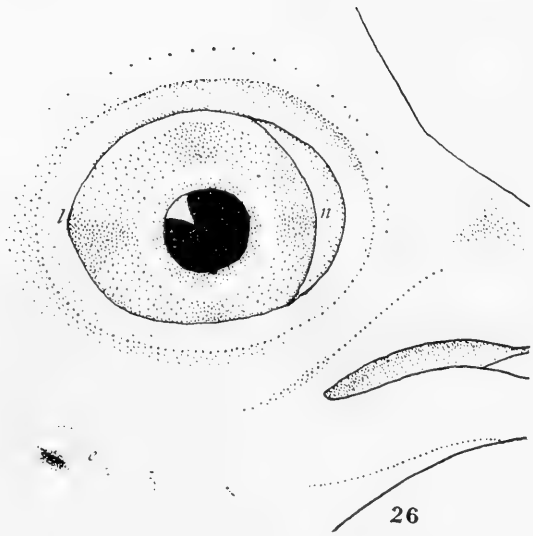


## PLATE 4

### EXPLANATION OF FIGURES

Very much enlarged camera-lucida drawings, showing the external parts of the eye. *c*, trace of the chorioid fissure; *e*, ear; *l*, margin of lids; *n*, nictitating membrane.  $\times 15$ .

- 24 Embryo at the age of  $7\frac{3}{4}$  days.
- 25 Embryo at the age of 8 days.
- 26 Embryo at the age of 9 days.
- 27 Embryo at the age of 10 days.



## PLATE 5

### EXPLANATION OF FIGURES

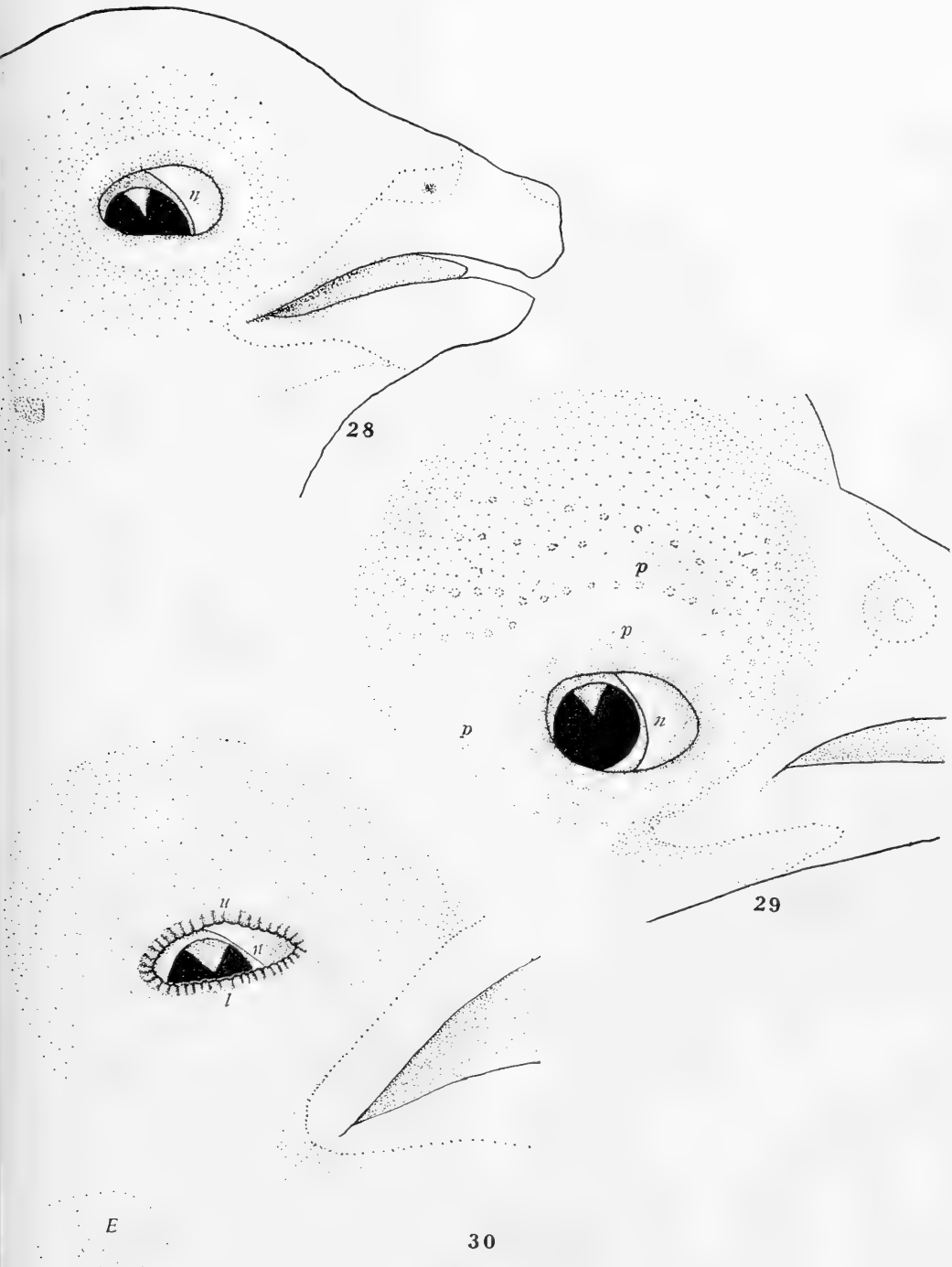
Showing advanced stages in the development of the external parts of the eye of the sparrow. *l*, lower lid; *n*, nictitating membrane; *p*, feather follicles; *u*, lower lid.  $\times 15$ .

28 Embryo at the age of 11 days.

29 Embryo at the age of 12 days.

30 Embryo at the age of 13 days, approximately the age of hatching. The margins of the lids show the convoluted appearance similar to that of the adult. The developing feather follicles in this figure have been omitted.



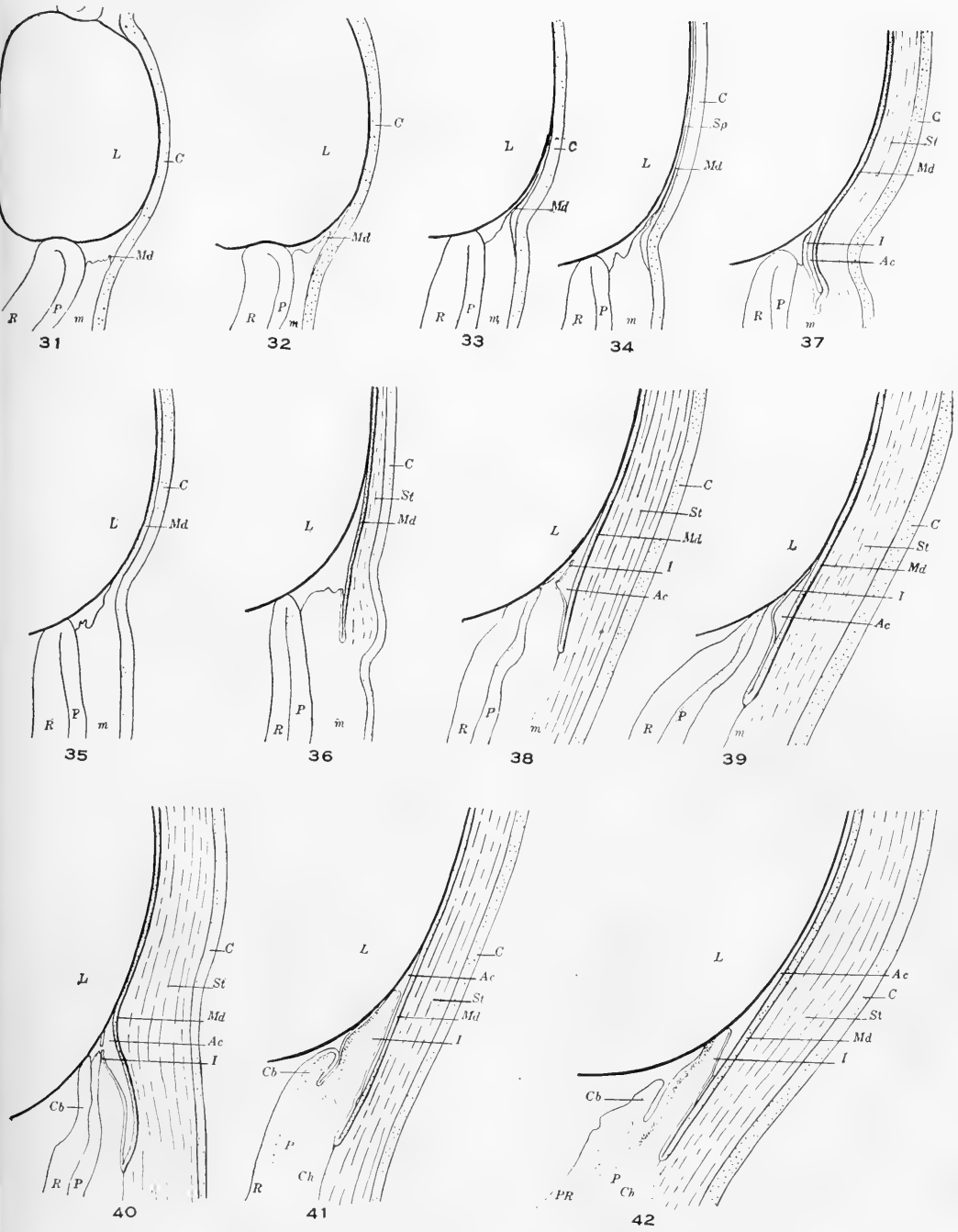


## PLATE 6

### EXPLANATION OF FIGURES

Outline drawings representing the development of the cornea, the iris, and the aqueous chamber of the sparrow eye at different ages. The drawings were made by tracing the outlines of sections projected on a screen by a stereopticon. All the figures are of the same magnification. *Ac*, aqueous chamber; *C*, conjunctival epithelium; *Cb*, ciliary process; *Ch*, chorioid; *I*, iris; *L*, lens; *m*, mesoderm; *Md*, membrane of Descemet; *P*, pigment portion of the retina; *PR*, pars ciliaris retinae; *R*, nervous portion of the retina; *St*, stratified portion of the cornea, the substantia propria.

- 31 Sparrow embryo at the age of 2 days' incubation.
- 32 Sparrow embryo at the age of 3 days' incubation.
- 33 Sparrow embryo at the age of 4 days' incubation.
- 34 Sparrow embryo at the age of 5 days' incubation.
- 35 Sparrow embryo at the age of 6 days' incubation.
- 36 Sparrow embryo at the age of 7½ days' incubation.
- 37 Sparrow embryo at the age of 8 days' incubation.
- 38 Sparrow embryo at the age of 9 days' incubation.
- 39 Sparrow embryo at the age of 10 days' incubation.
- 40 Sparrow embryo at the age of 11 days' incubation.
- 41 Sparrow embryo at the age of 12 days' incubation.
- 42 Sparrow embryo at the age of 13 days' incubation.



## PLATE 7

### EXPLANATION OF FIGURES

Microphotographs showing various stages in the development of the eye. *Al* and *Pl*, anterior and posterior portions of the lens; *Lc*, lens; *OpSt*, optic stalk; *Opv*, optic vesicle; *P*, pigment layer; *R*, retinal layer.

43 Section through the optic vesicles, *Opv*, and the optic stalks; *OpSt*, of the chick about forty hours old. The thickening of the epithelium, *L*, which later becomes the lens, is very marked.  $\times 80$ .

44 Section through the head of a fifty- to fifty-six-hour chick, showing the invagination of the ectoderm in the formation of the lens vesicle, *Lc*, and the pushing in of the optic vesicle to form the optic cup.  $\times 80$ .

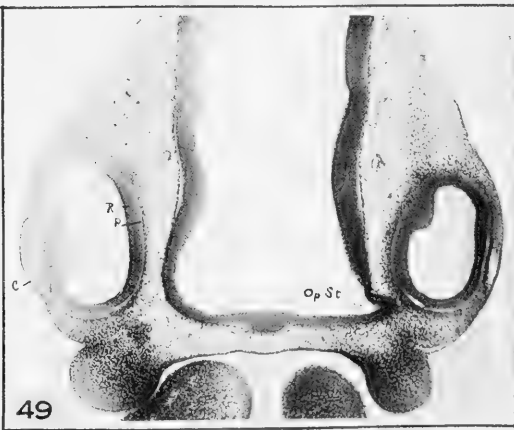
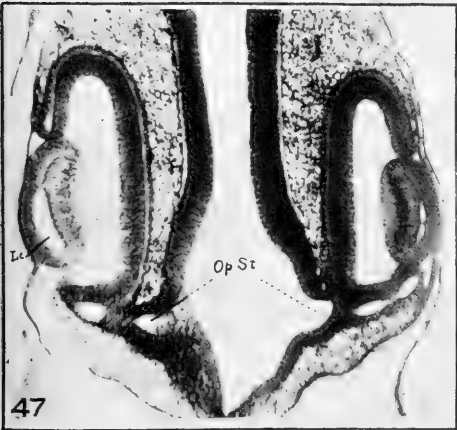
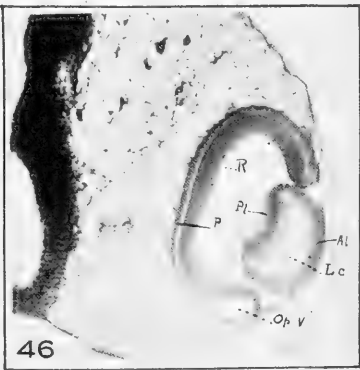
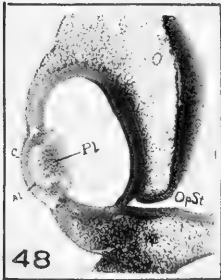
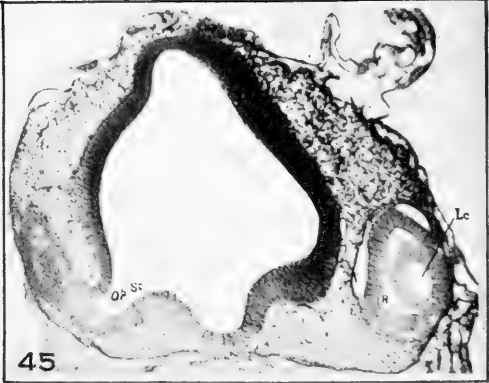
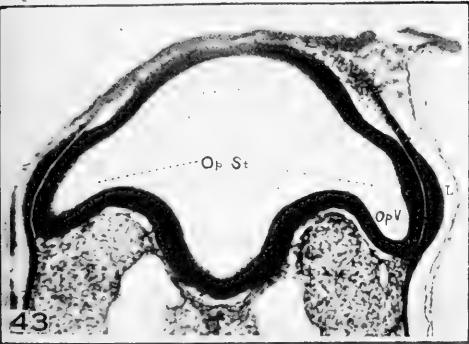
45 Section through the head of a sixty-four-hour chick. The lens vesicle, *Lc*, is completely formed and separated from the ectoderm. The retinal, *R*, and pigment, *P*, portions of the optic cup can be distinguished.  $\times 80$ .

46 Section through the head of a sixty-eight-hour chick. The lens vesicle, *Lc*, shows an increase in the posterior wall, *Pl*, over the anterior wall, *Al*. The optic cup with its retinal, *R*, and pigment, *P*, layers separated by a small portion of the primary optic vesicle, *Opv*, is seen.  $\times 80$ .

47 Section through the head of a 90- to 100-hour chick, showing the rapid growth of the cells of the posterior portion of the lens almost filling the lens vesicle, *Lc*. Portions of the optic stalk, *OpSt*, can also be seen.  $\times 80$ .

48 Section through the eye of a sparrow embryo at the age of two days, showing the optic stalk, *OpSt*, the corneal ectoderm, *c*, and the anterior, *Al*, and posterior, *Pl*, layers of the lens. The lens cavity has entirely disappeared.

49 Section through the eyes of a sparrow embryo at the age of two days passing through the center of the left eye. The ectoderm, *c*, the retinal, *R*, and the pigment, *P*, portions of the retina and a part of the optic stalk, *OpSt*, are seen.

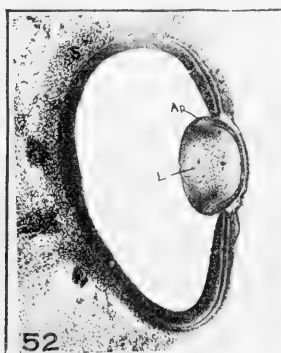
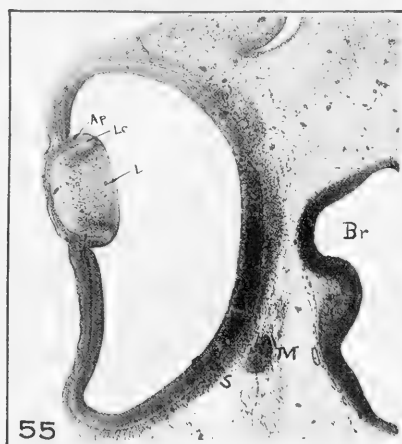
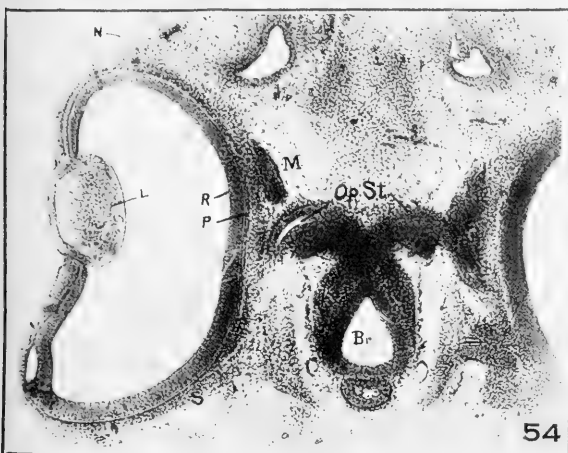
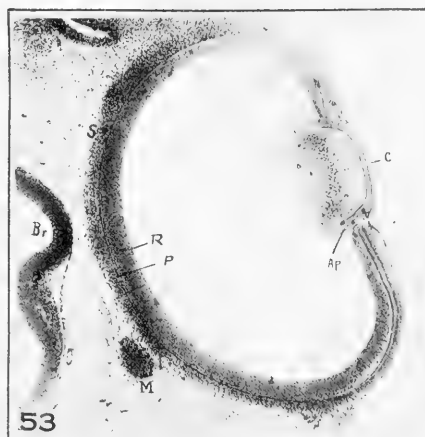
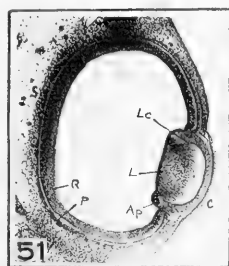
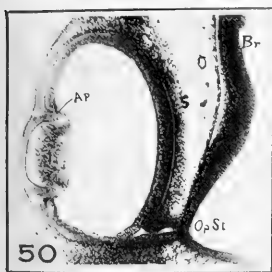


## PLATE 8

### EXPLANATION OF FIGURES

Microphotographs of horizontal sections of head, showing different stages in the developing eye of the sparrow. *Ap*, annular pad of lens; *Br*, brain cavity; *C*, cornea; *E*, ectoderm; *L*, lenticular portion of the lens; *Lc*, lenticular chamber appearing as a line or separation between the cells which have developed from the posterior and anterior surfaces of the lens vesicle; *Ld*, lid; *M*, developing eye muscles; *N*, nictitating membrane; *OpSt*, optic stalk; *P*, developing pigment layer, the posterior wall of the original optic vesicle; *R*, the anterior, or invaginated wall of the optic vesicle which is developing into the remaining layers of the retina; *S*, developing sclerotic and chorioid layers.

- 50 Left eye of embryo sparrow at the age of 3 days. × 40.
- 51 Right eye of embryo sparrow at the age of 3 days. × 40.
- 52 Right eye of embryo sparrow at the age of 4 days. × 40.
- 53 Right eye of embryo sparrow at the age of 5 days. × 40.
- 54 Left eye of embryo sparrow at the age of 5 days. × 40.
- 55 Left eye of embryo sparrow at the age of 6 days. × 40.
- 56 Right eye of embryo sparrow at the age of 7 days. × 40.



## PLATE 9

### EXPLANATION OF FIGURES

Microphotographs of horizontal sections through the head, showing different stages in the developing eye of the sparrow. *Ap*, annular pad of lens; *Br*, brain; *C*, cornea; *E*, ectoderm; *L*, lenticular portion of lens; *Lc*, lenticular chamber represented by a narrow line; *Ld*, lid; *M*, developing eye muscle; *N*, nictitating membrane; *ON*, optic nerve; *P*, pecten; *Pg*, pigment layer of the retina; *R*, remaining portion of retina; *S*, developing sclerotic and chorioid coats.

57 Right eye of embryo of  $7\frac{1}{4}$  days, through the center of the lens.  $\times 40$ .

58 Right eye of embryo of  $7\frac{1}{4}$  days, showing the early development of the nictitating membrane and lids.  $\times 40$ .

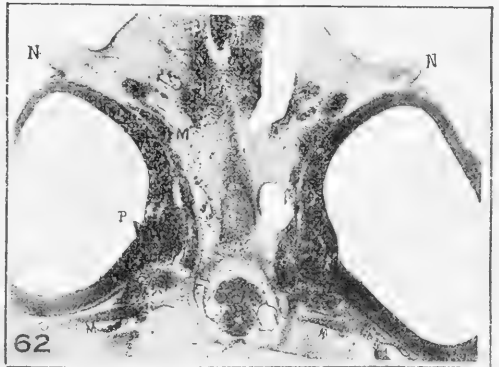
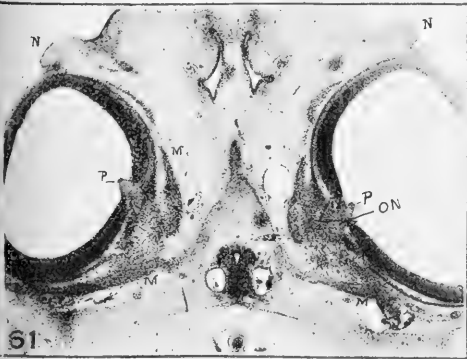
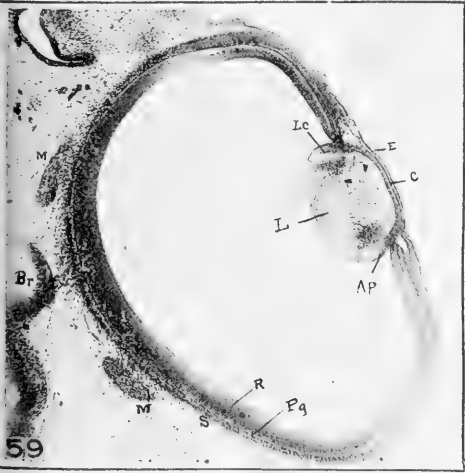
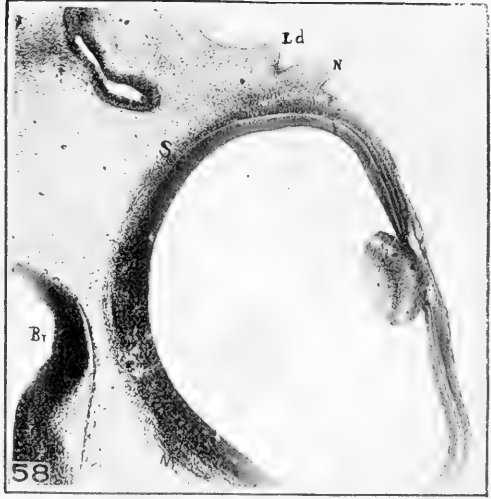
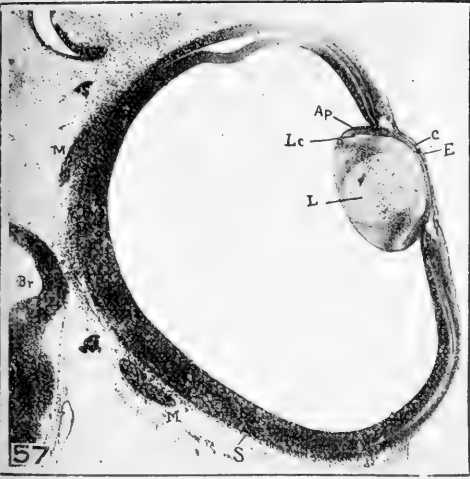
59 Left eye of embryo of  $7\frac{1}{4}$  days, showing the center of the lens. The different parts of the developing lens are very noticeable and the retina has increased in thickness. The anterior part of the eye is at the bottom of the figure.  $\times 40$ .

60 Embryo of  $7\frac{1}{4}$  days. Section passes through the nerve entrance and shows the first trace of the pecten.  $\times 40$ .

61 Embryo of  $7\frac{3}{4}$  days. Section passes through the nerve entrance and shows the pecten appearing as a conical projection, a little more advanced in development than in figure 60.  $\times 20$ .

62 Same series as figure 61, the section passing through head at a different level.  $\times 20$ .





## PLATE 10

### EXPLANATION OF FIGURES

Microphotographs of horizontal sections through the head of the sparrow, showing different stages in the development of the eye. *Ap*, annular pad of lens; *Br*, brain; *C*, cornea; *Ch*, chiasma; *Chr*, chorioid; *E*, ectoderm (epithelium); *L*, lenticular portion of lens; *Lc*, lenticular chamber represented by a line; *Ld*, lid; *M*, developing eye muscles; *N*, nictitating membrane; *Op*, optic nerve; *P*, pecten; *Pg*, pigment layer of retina; *R*, retina; *Scl*, sclera.

63 Embryo of  $7\frac{3}{4}$  days, showing the development of the nictitating membrane and lid.  $\times 20$ .

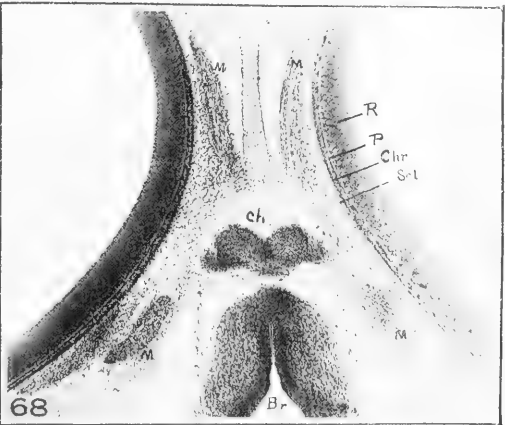
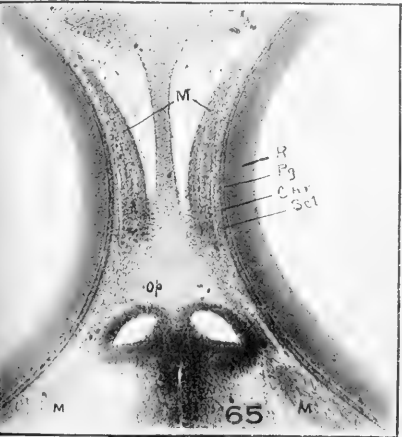
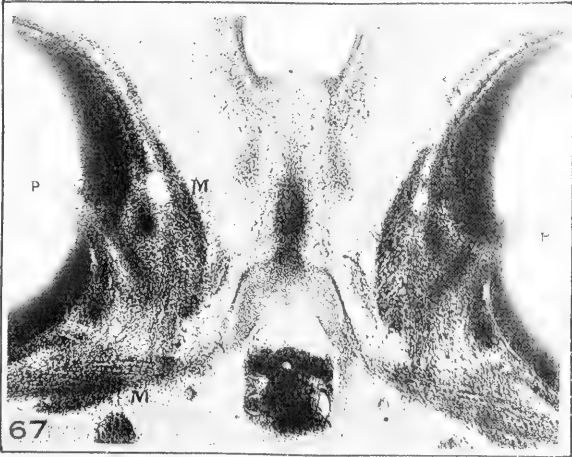
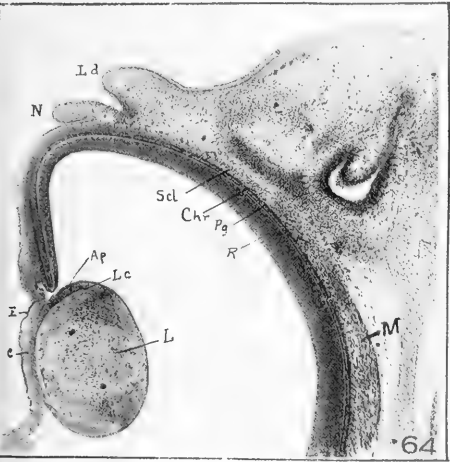
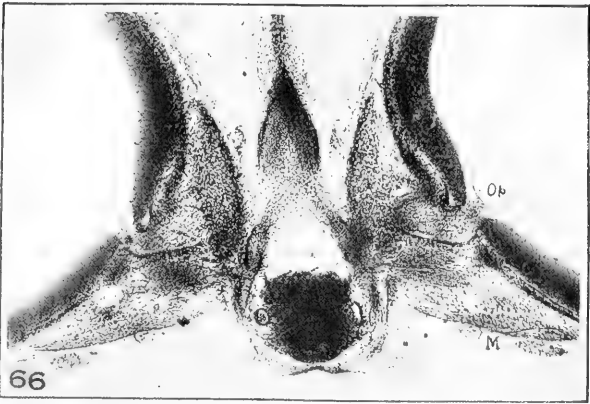
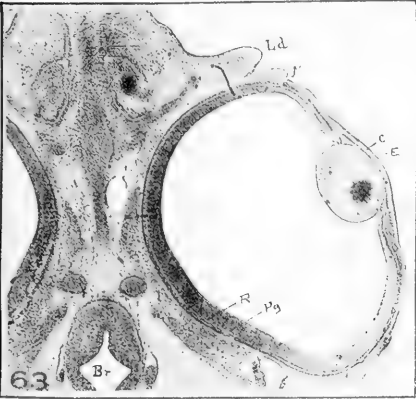
64 Embryo of 8 days. Shows advancement in development of nictitating membrane, retina, lens, and eye muscles.  $\times 40$ .

65 Same series as figure 64, but at a different level. A portion of the optic stalks close to the brain is seen at *Op*. The internal and external rectus muscles can be identified.  $\times 40$ .

66 Same series as figure 64 at the level of the dorsal edge of the optic nerve entrance.  $\times 40$ .

67 Same series as figure 64 through the most prominent part of the developing pecten. At this stage the pecten appears as a conical mass of cells arranged in the form of a ridge along the extent of the nerve entrance.  $\times 40$ .

68 Same series as figure 64 through the optic chiasm.  $\times 40$ .



## PLATE 11

### EXPLANATION OF FIGURES

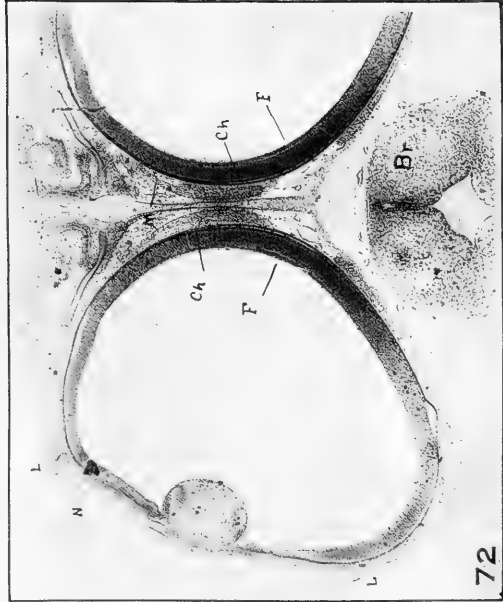
Microphotographs of horizontal sections through the head of the embryo sparrow, showing different stages in the development of the eye. *Br*, brain; *C*, cornea; *Ch*, chorioid; *F*, developing area and fovea centralis as indicated by the place of first differentiation of the retina into layers and by a thickening of the chorioid; *I*, iris; *L*, lids; *M*, eye muscles; *N*, nictitating membrane.

69 Section through the center of the eyes of an embryo 9 days old. The nictitating membrane and lid is well advanced and the first differentiation of the retina into layers appears over a small area at *F*. This region later becomes the area and fovea centralis. The chorioid is also thickened in this same region.  $\times 20$ .

70 Section through the head of an embryo 10 days old and passing through the region of the developing area and fovea. The layers of the retina are partially differentiated over the whole of the retina, but much more distinctly in the region of the fovea.  $\times 20$ .

71 Section of embryo 10 days old through the center of the developing area and fovea centralis. The layers of the retina are seen to be more sharply differentiated in this region than elsewhere. This section shows also how closely together the eyes are situated in the head. The iris is beginning to show distinctly.  $\times 20$ .

72 Section of embryo about 9½ days old passing through the center of the left eye and developing area and fovea. This shows an intermediate development between figures 69 and 71. The chorioid is seen to be perceptibly thicker in the region of the area and fovea centralis than in other parts of its extent.  $\times 20$ .



## PLATE 12

### EXPLANATION OF FIGURES

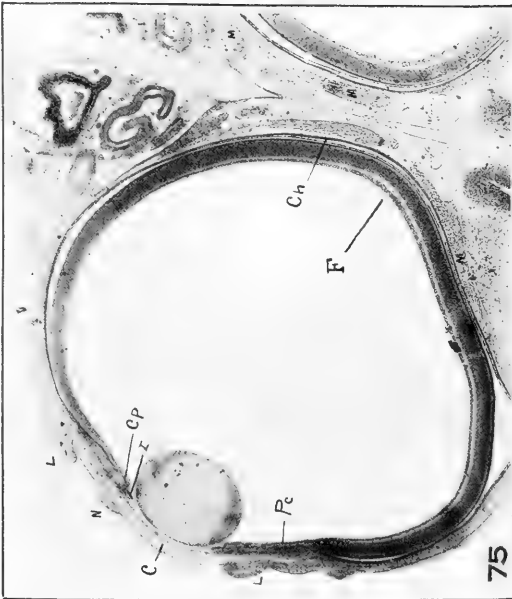
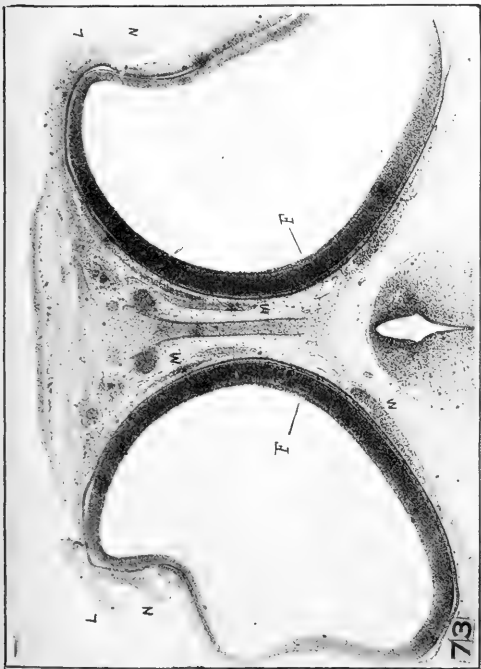
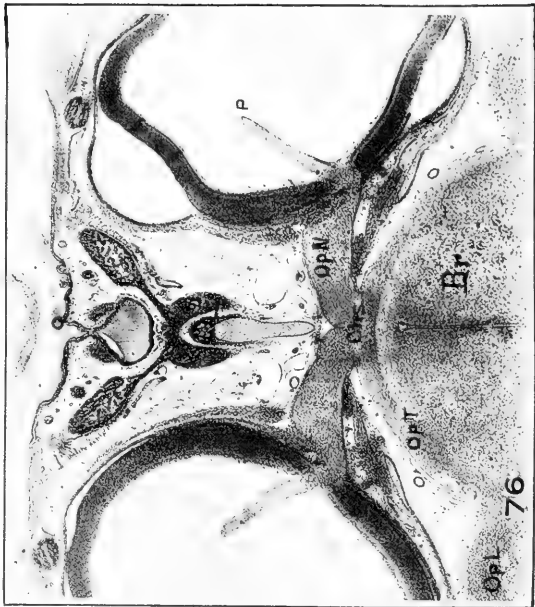
Microphotographs of horizontal sections through the head of the embryo sparrow, showing different stages in the development of the eye. *Br*, brain; *C*, cornea; *Ch*, choroid; *Cp*, developing ciliary processes; *Chs*, chiasma; *P'*, developing area and fovea centralis; *I*, iris; *L*, lids; *M*, eye muscles; *N*, nictitating membrane; *OpL*, optic lobe; *OpN*, optic nerve; *OpT*, optic tract; *P*, pecten; *Pc*, pars ciliaris retinae.

73 Section of embryo about 11 days of age passing through about the center of the area and fovea centralis.  $\times 20$ .

74 Same series as figure 73 passing through the pecten some distance below the nerve entrance. The conical shape of the pecten is still very noticeable.  $\times 20$ .

75 Section of embryo about 12 days old through the center of the area and fovea centralis, but slightly to one side of the center of the lens. The lids and nictitating membrane have grown over the front of the eye to quite an extent. All the layers of the retina except the rod-and-cone layer are distinguished under the microscope.  $\times 20$ .

76 Section of embryo about 12 days old through the pecten, optic nerves, chiasma and tracts. The pecten has become much more uniform in width, but shows more of the folds characteristic of the adult. It still consists of embryonic mesodermal tissue. A few small blood-vessels can be seen at the base under the microscope and a greater number near the free margin.  $\times 20$ .



## PLATE 13

### EXPLANATION OF FIGURES

Microphotographs of horizontal sections through the head of the embryo sparrow, showing different stages in the development of the eye. *Ch*, chiasma; *F*, developing area and fovea centralis; *L*, lids; *N*, nictitating membrane; *Op*, optic nerve; *P*, pecten.

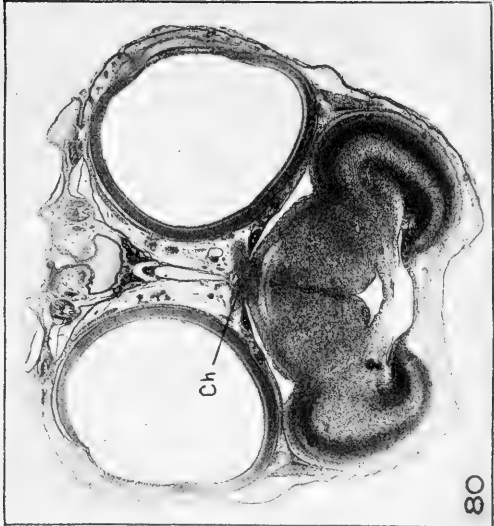
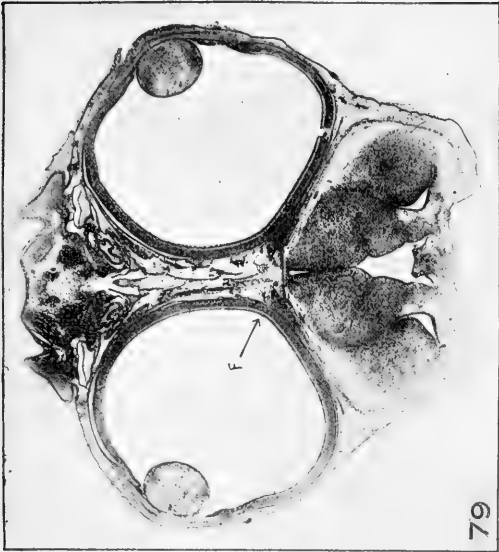
77 Section through the lower part of the eye of an embryo of 12 days parallel to the line *S-S* of figure 14. The extent of the pecten over the lower portion of the eye is noticeable, since the section passes through the base of the distal portion. Under the microscope small blood-vessels may be seen throughout this region, but most numerous in that portion near the nerve entrance.  $\times 40$ .

78 Section through the head of an embryo about 13 days old (the age of hatching); a little above the center of the eye and through the edge of the developing area centralis, to show the development of the lids and nictitating membrane. This section was made along the plane *S-S* of figure 15.  $\times 10$ .

79 Same series as figure 78 passing through the center of the developing area and fovea centralis and about the center of the lens.  $\times 10$ .

80 Same series as figure 78 passing at a lower level below the lens through the chiasma, but above the nerve entrance.  $\times 10$ .





## PLATE 14

### EXPLANATION OF FIGURES

Microphotographs of sections of the sparrow eye to show the development of the different structures. *Ap*, annular pad of lens; *C*, cornea; *Cp*, ciliary process; *F*, area and fovea centralis; *Hg*, Harder's gland; *I*, iris; *L*, lower lid; *Ll*, upper lid; *Lg*, lacrimal gland; *Op*, optic nerve; *OpT*, optic tract; *P*, pecten.

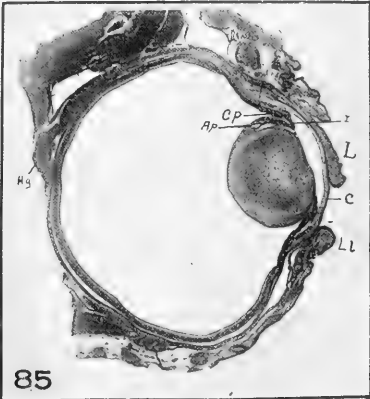
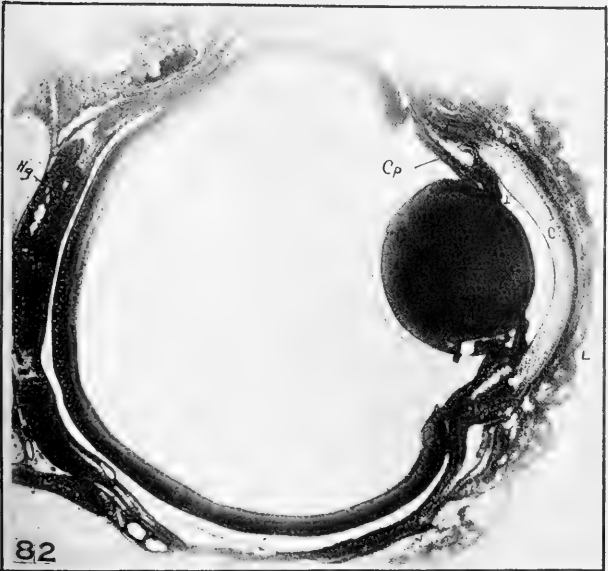
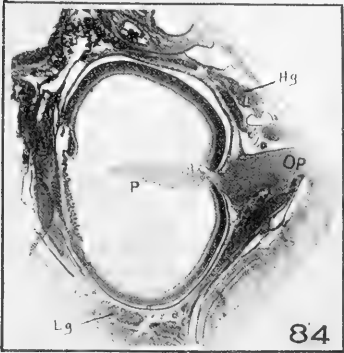
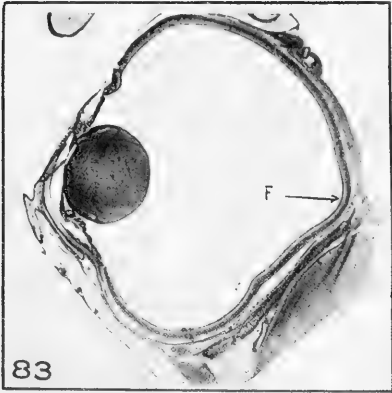
S1 Horizontal section through the head of an embryo at the age of 13 days (the age of hatching) along the plane S-S of figure 15. The relation of the optic tracts, chiasma, nerves, and pecten is shown. The pecten shows a more flattened appearance, having almost completely lost its conical shape, but is in no way folded.  $\times 10$ .

S2 Vertical section through the center of the right eye of a young sparrow two days after hatching and before the eyes have opened. The complete closure of the lids is noticed.  $\times 20$ .

S3 Horizontal section through the center of the left eye of a young sparrow 2 days after hatching. The fovea and area centralis show very little differentiation. There is a slight thinning of the retina at *F*. All the layers of the retina are more definite, but the rod-and-cone layer. These latter elements appear as slight projections from the outer nuclear layer.  $\times 10$ .

S4 Same series as figure S3 passing through the nerve entrance at a lower level. The pecten now shows some of the characteristic folds found in the adult. The layers of the retina are more sharply defined except the rod-and-cone layer.  $\times 10$ .

S5 Vertical section through the right eye of a young sparrow 4 days after hatching. The lids are now seen to be partly opened and the iris, the ciliary bodies, the annular pad, and chamber of the lens are well defined. The section passes a little to one side of the developing fovea. The rods and cones now appear as conical projections about one-fourth the length of the adult.  $\times 10$ .



## PLATE 15

### EXPLANATION OF FIGURES

Microphotographs of eyes of the young sparrow after hatching, showing various stages in the development of the different parts. *Ap*, annular pad of lens; *B*, bone of orbit; *C*, cornea; *Cp*, ciliary processes; *CScl*, cartilage of sclerotic coat; *F*, developing fovea; *Hg*, Harder's gland; *L*, lenticular chamber; *Ll*, lower lid; *Lu*, upper lid; *N*, nictitating membrane; *OpN*, optic nerve; *Or*, ora serrata; *P*, pecten.

86 Vertical section of the right eye of a young sparrow hatched 4 days passing through the center of the developing fovea and through the more distal portion of the nerve entrance. A few more blood-vessels are found in the pecten.  $\times 10$ .

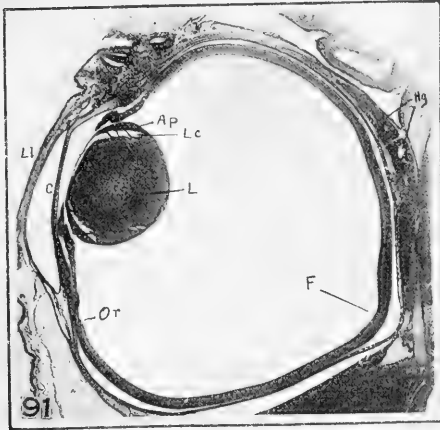
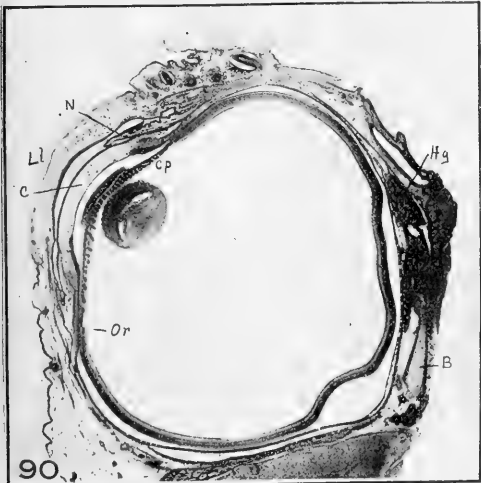
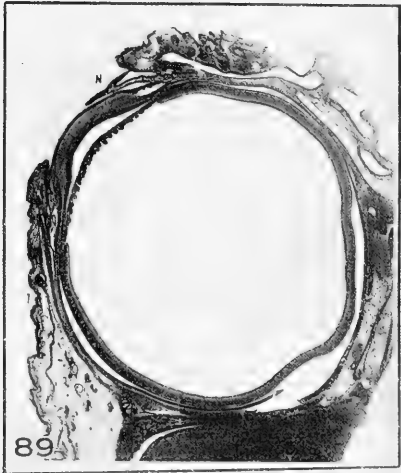
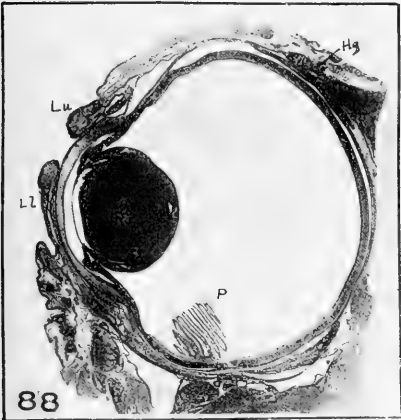
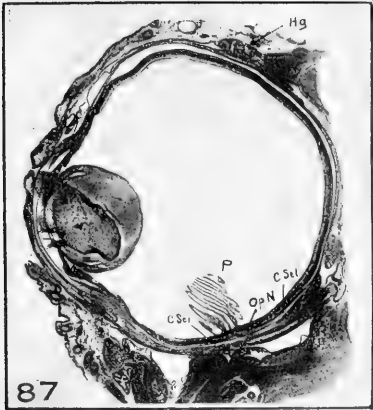
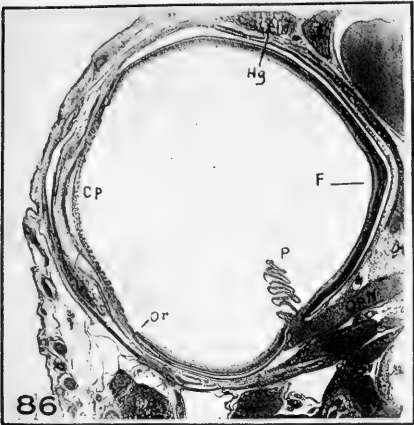
87 Same series as figure 86 through the distal end of the pecten and nerve entrance. A break in the cartilage layer of the sclera (appears as a light line, *CScl*) shows where the distal part of the nerve pierces the eye wall.  $\times 10$ .

88 Same series as figure 86 passing through the extreme distal end of the pecten beyond the last trace of the nerve entrance. The pecten and nerve entrance thus extend from a little below the center of the eye obliquely downward and forward almost to the ora serrata.  $\times 10$ .

89 Horizontal section of the left eye of a young sparrow 4 days old. This shows the nictitating membrane well developed. This section passes through the eye above the lens through the ciliary processes.  $\times 10$ .

90 Same series as figure 89 at a little lower level passing through the edge of the lens and through the thickened margin of the lower lid.  $\times 10$ .

91 Same series as figure 89 passing through the center of the lens and the center of the developing fovea. The retina at the fovea is seen to be slightly thinned. The layers of the retina are well defined except the rods and cones. These appear as conical projections with slender points having a total length of about one-fifth that of the adult. The ciliary muscles appear well striated at this age.  $\times 10$ .



## PLATE 16

### EXPLANATION OF FIGURES

Microphotographs of sections through the eye of the sparrow at different ages showing advancing stages in the development of the various structures. *Ap*, annular pad of lens; *C*, cornea; *Ch*, chorioid; *Chs*, chiasma; *Cp*, ciliary processes; *Cm*, ciliary muscles; *Hg*, Harder's gland; *I*, iris; *L*, lenticular portion of lens; *Lc*, lenticular cavity; *Ld*, lids; *Lg*, lacrimal gland; *M*, eye muscles; *N*, nictitating membrane; *OpN*, optic nerve; *Or*, ora serrata; *P*, pecten.

92 Horizontal section of the left eye of a young sparrow 4 days after hatching through the center of the lens.  $\times 10$ .

93 Same series as figure 92 through the nerve entrance and folds of the pecten. At this age the pecten has eighteen folds, this is only two less than in the adult. The blood-vessels, however, are not well formed as in the adult.  $\times 10$ .

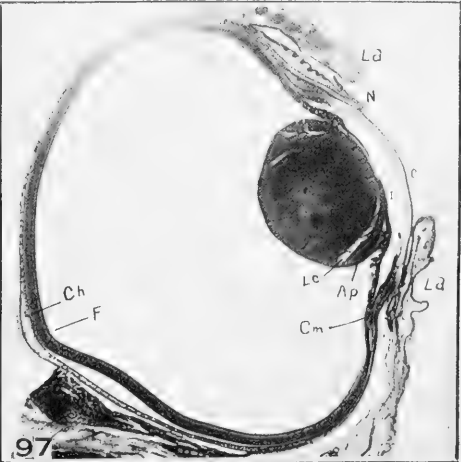
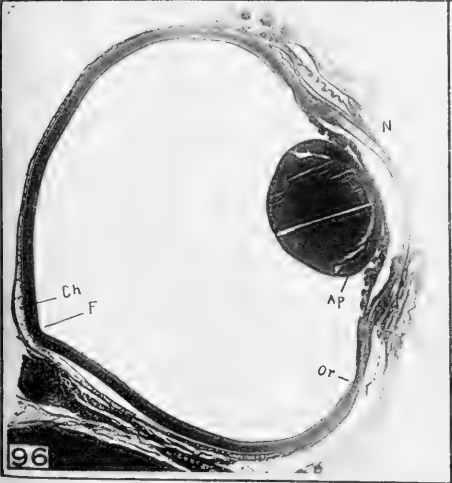
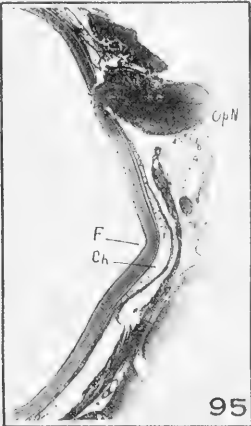
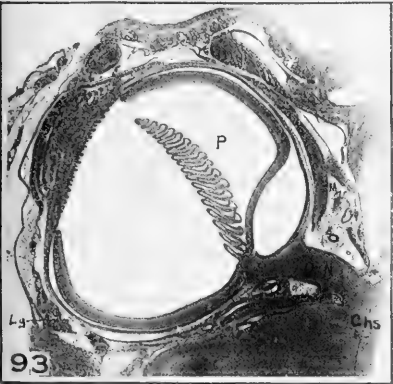
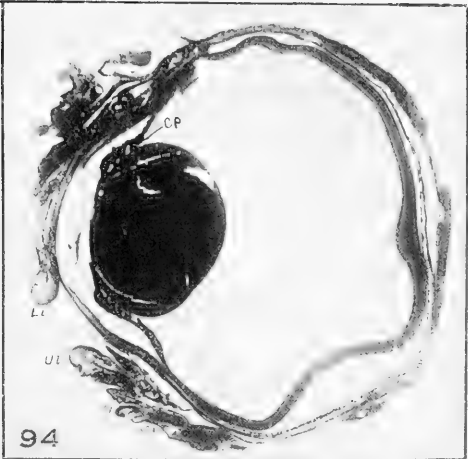
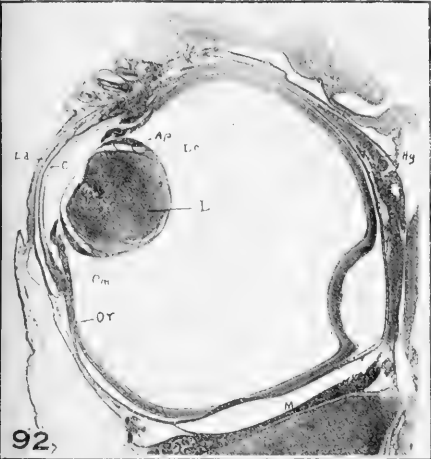
94 Vertical section of the eye of a young sparrow about 5 or 6 days after hatching.

95 Posterior portion of the eye of the same series as figure 94, passing through the nerve entrance and the center of the developing fovea. The very noticeable thickening of the chorioid is seen just back of the fovea. The fovea is pitting in slightly and the retinal layers are beginning to thin out somewhat in the immediate center. The rods and cones have reached almost their adult length and the pigment layer is sending processes between the outer segments of the rods and cones.  $\times 10$ .

96 Horizontal section through the eye of a young sparrow of about 6 days after hatching, passing through the center of the developing fovea, but to one side of the center of the lens. The zonula fibers are seen.  $\times 10$ .

97 Same series as figure 96 passing through the edge of the pupil.  $\times 10$ .

98 Same series as figure 96 passing through the center of the lens.  $\times 10$ .



# PLATE 17

## EXPLANATION OF FIGURES

Microphotographs of sections through the eye of the young sparrow, showing the development of the various structures. *Ap*, annular pad of the lens; *Br*, brain; *C*, cornea; *Ca*, thickened portion of the cartilage of the sclerotic coat which surrounds the optic nerve entrance; *Ch*, chorioid; *Chs*, chiasma; *Cp*, ciliary processes; *C'g*, lacrimal ducts; *F*, developing fovea; *Hg*, Harder's gland; *I*, iris; *L*, lenticular portion of the lens; *Lc*, lenticular chamber; *Ld*, lids; *Lg*, lacrimal gland; *M*, eye muscles; *N*, nictitating membrane; *OpN*, optic nerve; *Or*, ora serrata; *P*, pecten; *Q*, quadratus muscle; *SclP*, scleral plates; *T*, tendon of pyramidalis muscle passing through the loop of the quadratus.

99 Horizontal section through the right eye of a young sparrow about 6 days after hatching. Section a little to one side of the center of the lens. The connection of the lacrimal gland with the posterior angle of the eye is seen.  $\times 10$ .

100 Same series as figure 99. Section passes almost through the center of the lens and the tip of the proximal, or central end of the pecten.  $\times 10$ .

101 Same series as figure 99. Section passes through the optic nerve entrance and shows some of the folds of the pecten that lie in this plane. The thickened scleral cartilage is seen on either side of the nerve entrance.  $\times 10$ .

102 Horizontal section of the right eye of a sparrow about 6 days after hatching.

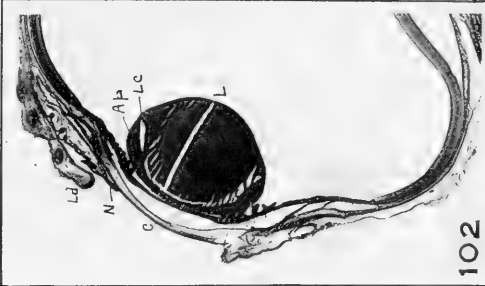
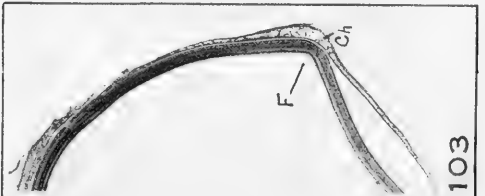
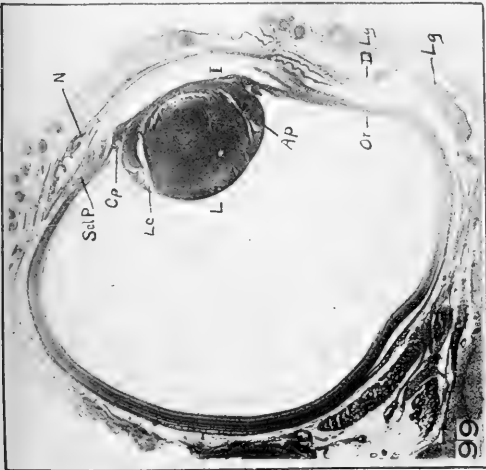
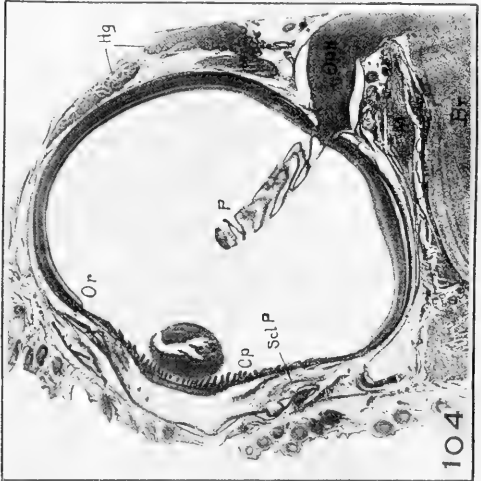
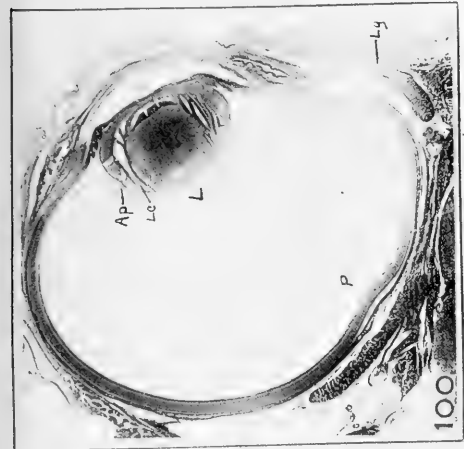
103 Same series as figure 102 passing through the center of the developing fovea. The thickening of the chorioid back of this region is very marked. The retina has separated from the chorioid in hardening, yet one can see a slight pitting in and thinning of the retina at this point.  $\times 10$ .

104 Same series as figure 102 passing through the nerve entrance and a portion of the pecten.  $\times 10$ .

105 Vertical section of the left eye of a young sparrow about 9 or 10 days after hatching. Section passes to one side of the lens, but through the center of the fovea and the optic nerve a little distance from its proximal entrance.

A cross-section of the tendon from the pyramidalis, surrounded by a loop of the quadratus muscle, is seen. A very marked thickening of the chorioid back of the fovea, the pitting in of the retina to form the fovea accompanied by a slight thinning of the retinal layers is shown.  $\times 10$ .





Resumen por el autor, William Harold Leigh-Sharpe,  
Londres.

La morfología comparada de los caracteres sexuales secundarios  
de los peces elasmobranquios—los órganos copuladores  
y sus sifones y glándulas. Segunda Memoria

Los órganos copuladores funcionan como penes y debieran ser considerados como tales órganos, y aunque indudablemente ambos son introducidos al mismo tiempo, pudiera suceder que uno solo fuese suficiente en algunas ocasiones. Los sifones de *Galeus* y *Mustelus* están enormemente desarrollados. *Lamna* posee una glándula copuladora especial que no es semejante a la de *Raja*, y la glándula copuladora de *Rhina* es un órgano mucoso, del mismo modo que el “órgano” labial. Las glándulas copuladoras se desarrollan de un modo semejante al de las glándulas prostáticas y son homólogas de estas últimas. Los órganos copuladores provistos de sifones poseen soportes esqueléticos resistentes y están provistos de dentículos que hacen su superficie áspera, impidiendo su deslizamiento; no dependen de la erección.

Los órganos copuladores provistos de glándulas accesorias son lisos, poseen mucho tejido erétil y soporte esquelético débilmente desarrollado; su deslizamiento está impedido por el rhipidion, muy desarrollado. El autor indica la existencia en alguna parte de la glándula, más probablemente en su pared muscular, de una secreción de un metabolito capaz de provocar la dilatación vascular. De este modo, las glándulas son fisiológicamente semejantes a las glándulas prostáticas. La glándula rectal no es una glándula mucosa, sino probablemente excretora.

Translation by José F. Nonidez  
Cornell Medical College, New York

## THE COMPARATIVE MORPHOLOGY OF THE SECONDARY SEXUAL CHARACTERS OF ELASMOBRANCH FISHES

THE CLASPERS, CLASPER SIPHONS, AND CLASPER GLANDS

MEMOIR II

W. HAROLD LEIGH-SHARPE

FIFTEEN TEXT FIGURES

The preceding memoir, which appeared in the *Journal of Morphology*, volume 34, page 245, contained a general introduction to the subject, a list of references, and a consideration of *Scyllium catulus*, *Scyllium canicula*, *Acanthias vulgaris*, and *Raia circularis*. In it the following nomenclature was adopted. The term siphon was given to a muscular sac ending blindly anteriorly, and opening posteriorly near the proximal end of the clasper. Its cavity is the siphon sac. In *Raia*, and as will presently be seen in *Lamna* also, the sac is almost filled by a gland—the clasper gland. The apopyle was defined as the proximal or anterior entrance to the clasper groove, or tube, and the hypopyle as the distal, or posterior exit therefrom. The parasiphons are, in *Scyllium*, a pair of similar and similarly situated sacs to the siphons, though much smaller and probably vestigial in function. The siphons and parasiphons debouch by a common exedra.

The rhipidion is a fan-like expansion near the distal extremity of the clasper, posterior to the hypopyle, attaining a greater development in *Raia* and *Galeus*, whose function is partly to spread the ejected spermatozoa in a radiating manner and partly for attachment during copulation. To these terms some new ones, such as pseudosiphons and perae, will now be added and defined in their proper places.

The present memoir deals with the following species:

1. <i>Galeus vulgaris</i> .....	360
2. <i>Mustelus vulgaris</i> .....	365
3. <i>Lamna cornubica</i> .....	367
4. <i>Rhina squatina</i> .....	373

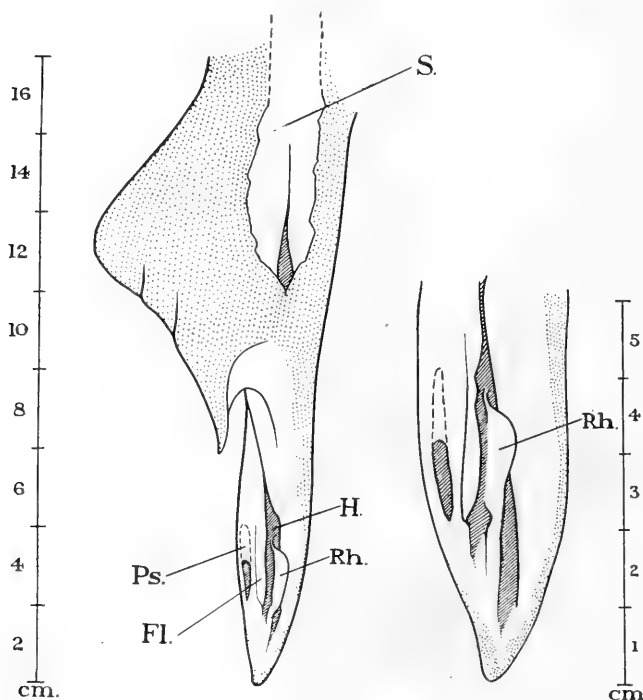


Fig. 1 *Galeus vulgaris*, with an enlarged view of the terminal portion of the clasper to the right. S., siphon; H., hypopyle; Ps., pseudosiphon; Fl., flap; Rh., rhipidion.

#### GALEUS VULGARIS

##### *The toper or tope*

In both *Galeus* and *Mustelus* the siphons attain enormous dimensions. In the specimen of *Galeus vulgaris* taken at Plymouth in July, 1918, upon which the following observations

were made, and which measured 80 cm.<sup>1</sup> from the tip of the snout to the posterior ends of the claspers, the siphons were 28 cm. in length, so that they cannot be included in their entirety in the figure (fig. 1).

The claspers, measuring about 8 cm. in length, are considerably longer than the pelvic fins. The clasper groove is open as in *Raia* since its edges do not approximate. As in *Raia* also the claspers are practically devoid of dermal denticles, being smooth to the touch, and composed of plenty of erectile tissue.

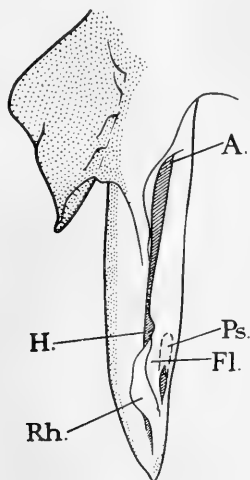


Fig. 2 *Galeus vulgaris*, dorsal aspect. A., apophyle; H., hypophyle; Fl., flap; Rh., rhipidion; Ps., pseudosiphon.

The rhipidion is, in this genus, more developed than in any so far considered, and is abundantly supplied with blood vessels, some pronounced superficially but omitted in the figure.

Toward the posterior end of the clasper, upon about the same level as the rhipidion, and on its inner side (since the clasper is twisted in the figure), is a small, blindly ending sac whose wide aperture points in a posterior direction. In naming this the

<sup>1</sup> This standard of measurement has been adopted in this and the following cases, since the animals were consigned, for the sake of convenience, to me without their tails.

pseudosiphon it is not implied that it is homologous, or even physiologically analogous with the siphon proper. As in *Raia* under similar circumstances, the apopyle exists rather as a region than as an aperture. A flap separates the hypopyle from the opening of the pseudosiphon. Slight vestiges of pseudosiphons may be seen in some specimens of *Scyllium canicula*.

The superficial walls of the siphon sac exhibit purple reticulations like the rhipidion, indicating that they are highly vascular. This is one of the reasons why this siphon was chosen for sectioning.

The detailed structure of the wall of the siphon is, in the main, the same as that of *Scyllium canicula*, and may be taken as a typical example (figs. 3 and 4). The epithelium is stratified and appears typically to comprise two rows of cells, as in *Scyllium*. Occasionally large mucus-secreting cells, each with a conspicuous nucleus, are seen. These would account for the minute amount of mucilage found in all siphons. These cells are characteristic and do not altogether resemble ordinary goblet cells, such as appear in *Rhina*. They are, however, well filled with mucus, which displaces the nucleus and cytoplasm to one side and is not stained by the ordinary dyes. Below the epidermis is a subepidermal layer of connective tissue, extremely rich in blood-vessels (fig. 3) and composed of spindle-shaped cells.

An acquaintance, who was present at the annual tope-fishing competition at Dover in the summer of 1919, was able to obtain, by imitating the means used by the competitors, some topes which, though maimed, he managed to keep alive for some days in tanks which had been previously prepared for their reception. I was able to inspect these topes which were in a condition of sexual desire. In the absence of females, it was noticed the males attempted to copulate with each other, though one ingenious observer considered that they were fighting, and another that they were attempting to relieve each other of copepod clasper and cloacal parasites (*Lernaeopoda galei* and *L. bidiscalis*), which, one would think, they could more effectively have done against the rocks, etc., provided at the bottom of the cistern. After the introduction of females, one pair was

killed in copula, by first stranding them and then administering a sharp blow on the snout with a mallet. These animals were preserved and dissected by me. Both claspers were inserted simultaneously, the rhipidion performing the function of fixation.

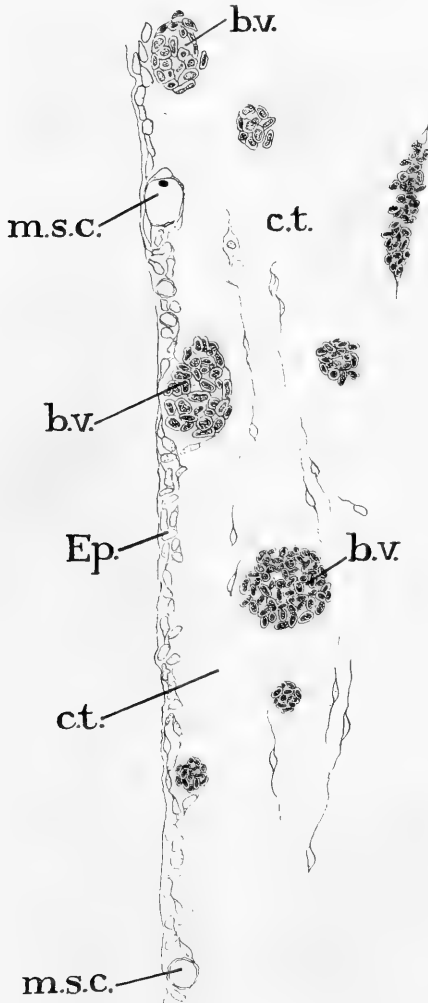


Fig. 3 *Galeus vulgaris*, a transverse section of the wall of the siphon under a high magnification; 5 $\mu$ . picro-formol, alum-carmin. *Ep.*, epidermis; *c.t.*, sub-epidermal connective tissue; *b.v.*, blood-vessels; *m.s.c.*, mucus-secreting cell.

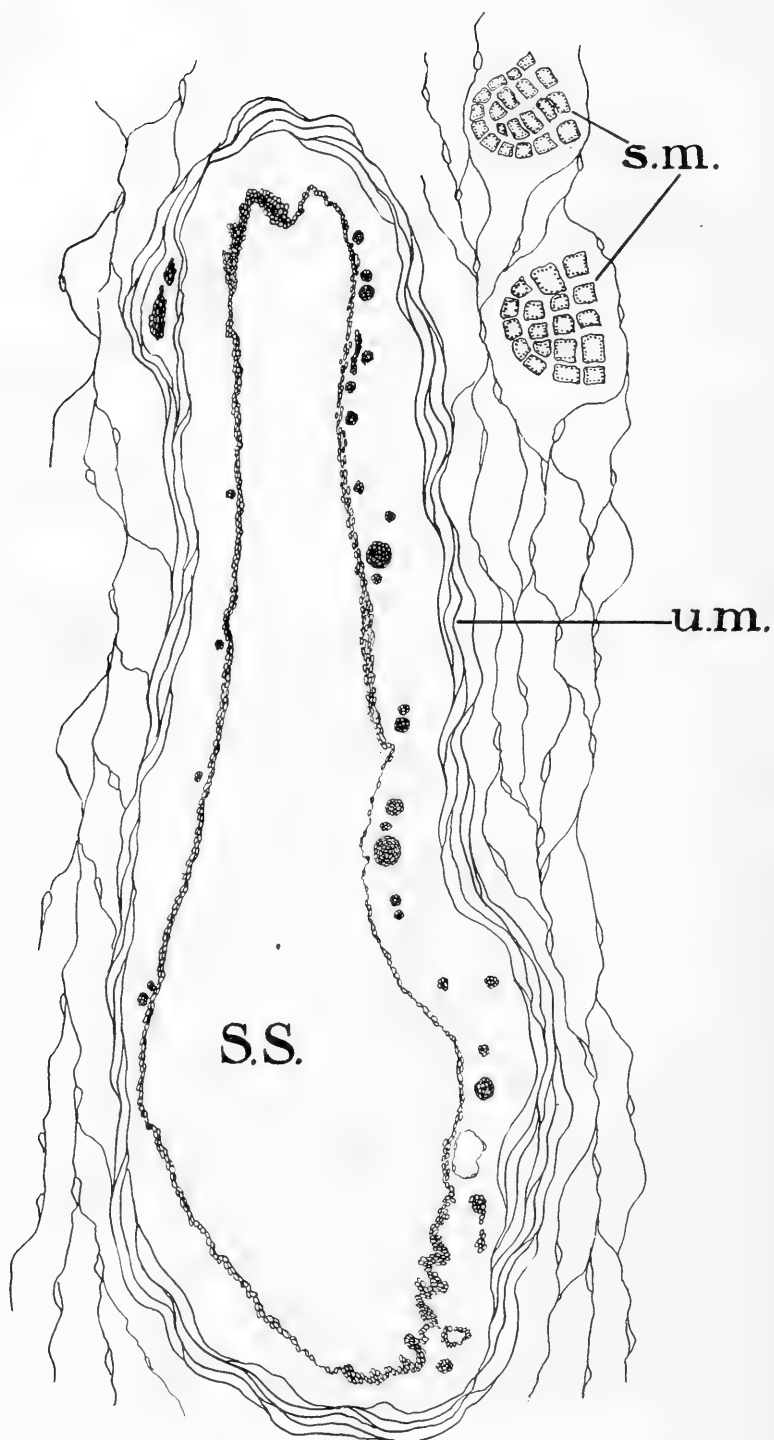


Fig. 4 *Galeus vulgaris*, transverse section of the siphon, the wall of which is shown magnified in figure 3. *S.S.*, siphon sac; *s.m.*, striped muscle; *u.m.*, unstriped muscle.



There are certainly two, probably three and possibly four breeding seasons in a year. Speaking from my own experience of *Scyllium canicula* from Plymouth and Bournemouth, the seasons are: 1) April to May; 2) July to August. Whether the season May to June is an extension of number (1) I am in doubt, and of a season in January should like further corroboration. The indications of the breeding season are: 1) Spermatozoa oozing from dead males on pressure of the finger on the urino-genital sinus; 2) spermatozoa oozing from dying or maimed Dover topes; 3) the presence of ova in the oviducal gland about to be coated with a 'shell' case. Breeding seasons for the same species may vary with, 1) weather; 2) locality, both factors being modified by temperature.

I was interested to notice that on handling a vigorous, strongly secured wounded male tope I could feel pulsations which presumably were those of the siphon. Spermatozoa did not in this case spurt from the clasper tip, but from the cloaca. On bending the claspers forward some ejection from the tip was obtained, but slight because the clasper is not a scroll-tube as in *Scyllium*, whereas in copula the oviducal wall would help to form a closed passage. Moreover, the tope must have been pumping spermatozoa with air, since its siphons had long been emptied of water in its struggles.

#### MUSTELUS VULGARIS

##### *The smooth-hound*

In *Mustelus* the siphons attain even larger dimensions than in *Galeus*, extending almost as far forward as the pectoral fins. In the specimen upon which the following observations were made, taken at Plymouth in July, 1918, and which measured 54 cm. from the tip of the snout to the posterior ends of the claspers, the siphons were 23 cm. in length, so that they cannot be included in their entirety in the figure (fig. 5).

As in *Galeus*, the claspers project considerably beyond the pelvic fins posteriorly. The clasper tube is closed for the greater part of its length, the edges overlapping in a scroll-like manner

as in *Scyllium*. There are therefore a distinct apophyle and hypophyle. The rhipidion is well developed. The distal end of the clasper is jointed, so that, by the aid of muscular contraction, the terminal portion of the clasper can be flexed abruptly outward, thus affording a powerful means of attachment during copulation, analogous with the spur already described in *Acanthias*. A hint of such an outward flexure can be seen occasion-

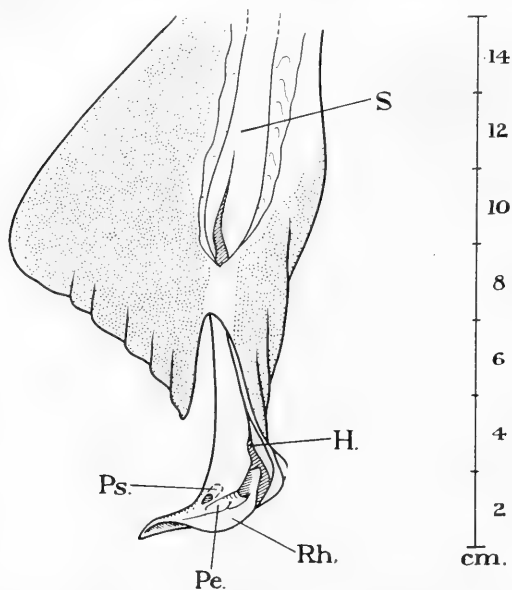


Fig. 5 *Mustelus vulgaris*. *S.*, siphon; *H.*, hypophyle; *Rh.*, rhipidion; *Ps.*, pseudosiphon; *Pe.*, perä.

ally in *Scyllium canicula*, although I am much in doubt whether such a modification is characteristic of *Scyllium*, since about 8 per cent of the individuals that come under my notice have the distal ends more markedly developed toward a spoon-shaped conformation, and these alone seem capable of such a flexure. Toward the posterior end of the clasper, at about the middle length of the rhipidion, upon its inner border, but coming to lie upon its outer border owing to natural torsion, is a small blindly ending sac, the pseudosiphon, whose aperture points in a pos-

terior direction. The pseudosiphon is not so large as in *Galeus*. On the outer side of the rhipidion and between it and the pseudosiphon is another similar sac, the pera, whose aperture faces forward confluent with the hypopyle.

The pera is apparently formed by the folding back of a flap like that of *Galeus* (which separates the rhipidion from the pseudosiphon) and its fusion with the base of the rhipidion. The use of the pera is problematical: it would appear to be disadvantageous, since half the spermatozoa which do not flow out on the other side of the rhipidion would enter the pera, and there collect. While vestiges of pseudosiphons may occasionally be seen in *Scyllium canicula*, no traces of perae can be observed.

#### LAMNA CORNUBICA

##### *The porbeagle*

This animal, coming as it does under the vulgar appellation of 'shark,' presents very peculiar and interesting features, for, in regard to the points I am considering, it approximates not to the Selachoidi, but to the Batoidei. Instead of possessing siphons similar to its congeners, the sac homologous with those structures is small (about 2 cm.) and, as in *Raia*, is almost completely filled by a gland—the clasper gland.

The claspers, too, are minute for so large an animal, for, in the porbeagle upon which the following observations were made, captured at Plymouth in July, 1918, and which measured 57 cm. from the tip of the snout to the posterior ends of the claspers, they are but 4 cm. in length. Moreover, they are little longer than the pelvic fins, so that they appear to project scarcely at all. *Scyllium canicula* is the only type under observation so far in which the claspers are not quite as long as the pelvic fins. Further, the clasper groove is not closed, is soft and devoid of dermal denticles, in all these particulars again resembling *Raia*. The rhipidion is pronounced as in *Raia*, and accessory structures such as pseudosiphons or perae are absent. The apopyle and hypopyle do not exist as apertures, but merely as local indication (fig. 6).

The clasper gland, however, does not precisely resemble that of *Raia* in being limited to the dorsal side of the siphon sac. The gland (fig. 7) completely surrounds the siphon, and is divided into numerous separate components, which almost entirely fill the cavity. The components are compact masses of tissue, not penetrated by ducts, as in *Raia*, but separated from one another by connective tissue.

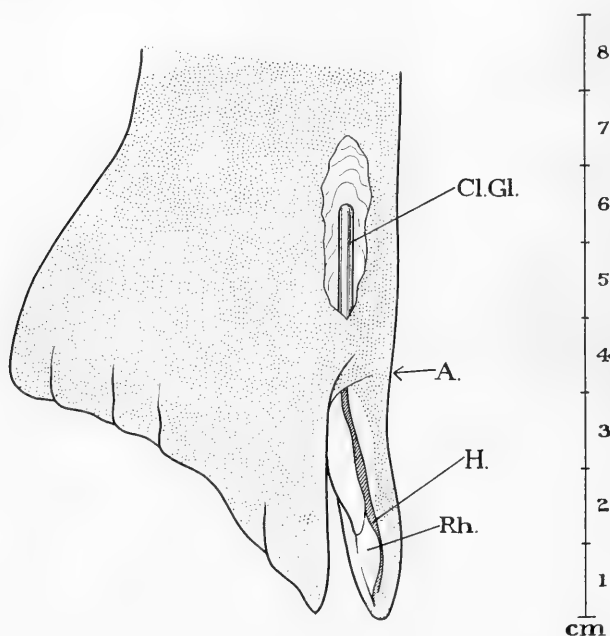


Fig. 6 *Lamna cornubica*. *Cl.Gl.*, clasper gland; *A.*, apopyle; *H.*, hypopyle; *Rh.*, rhipidion. Immature specimen.

The individual cells are not, as in *Raia*, spherical or cubical with large spherical nuclei nearly filling the cell, but are exceedingly long and spindle shaped, with elongated nuclei, and fitting into one another in a way that suggests non-striped muscle cells (fig. 8, B).

The pointed ends of the cells are toward the lumen of the gland. Around the lumen, forming an epithelium as it were, the spindle-shaped cells give way to a type with a flattened outer

border somewhat resembling columnar cells, with nuclei not quite so elongated; no doubt they are a modification of the spindle-shaped cells, and suggest by their appearance that they may secrete mucus, without being actually what are termed mucus-secreting cells in *Galeus*. No evidences of mucus are discernible, neither in the cells themselves nor in the lumen of the duct.

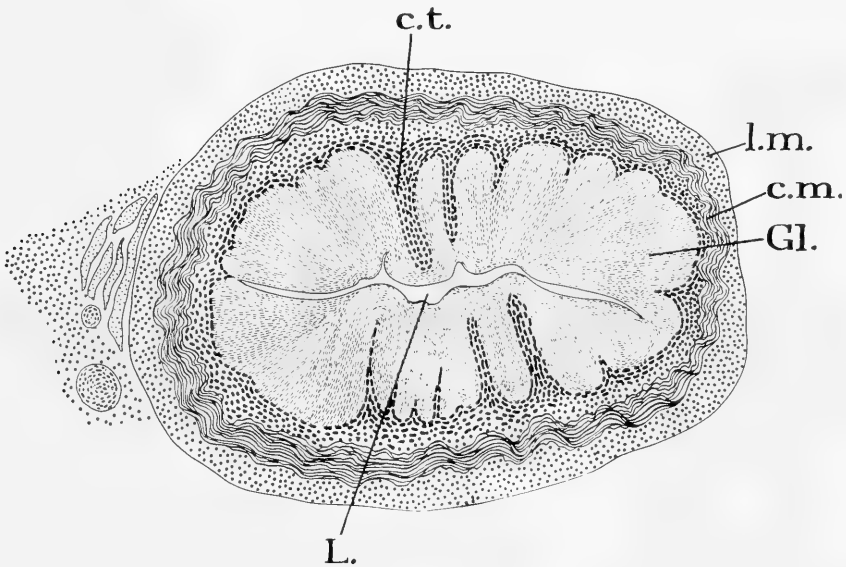


Fig. 7 *Lamna cornubica*, transverse section of siphon and gland. *l.m.*, longitudinal muscle; *c.m.*, circular muscles; *c.t.*, connective tissue; *Gl.*, gland; *L.*, lumen.

A recent theory in human physiology may receive some corroboration from the investigation of the clasper glands. The fundamental principle is that there are no such nerves as vasodilators, but that inhibition of the plain muscle of arterioles is really produced by the agency of a chemical by-product, or metabolite, originated either in the neighboring muscle or a neighboring gland.

The large amount of erectile tissue in the penis, and its obvious control by the erigens nerve, whereas there is comparatively

little muscle or gland in the organ, may be explained by the fact that the erigens nerve is also distributed to the prostate gland as well as to the other pelvic viscera. The fibers going to the penis are to be regarded as simply sensory in function, the real control of the vasodilator effect being due to the secretion of a special inhibitory substance by the prostate gland at the instigation of the erigens nerve. Barrington has, however, shown that this is not a true secretion, but is due to the pressing out of a secretion

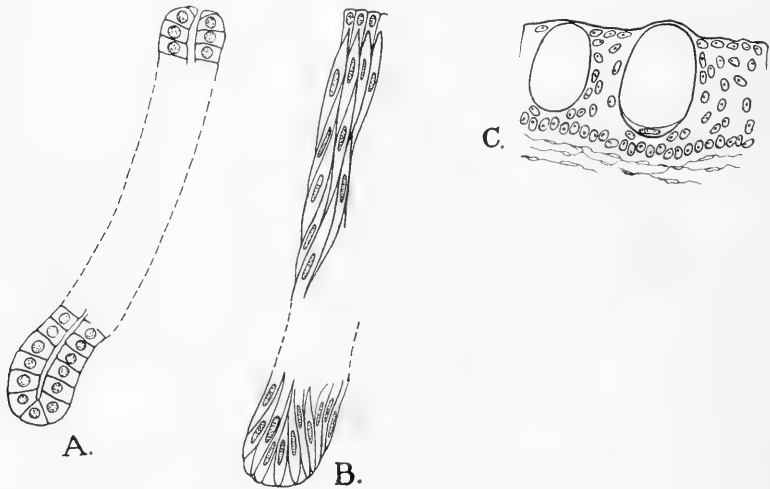


Fig. 8 A comparison of the typical cells of clasper glands under a high magnification. A, Raia; B, Lamna; C, Rhina.

owing to the contraction of some of the muscles surrounding the prostate gland. He has pointed out that a squeezing of the gland takes place both when the pelvic and the hypogastric nerves are stimulated, from which he concludes that the muscle fibers round the gland are of two kinds, partly belonging to the cloacal muscles, partly to the urodermal group.<sup>2</sup>

The bearing which this investigation appears to have upon this theory is that, of the fishes so far examined, those with the best-developed clasper glands are also the ones with the most

<sup>2</sup> Barrington, The variation in the mucin content of the bulbo-urethral glands. *Internat. Monatschr. f. Anat. u. Phys.*, Bd. 30, 1913.

erectile tissue in the clasper and the least amount of cartilaginous skeletal support; conversely, those species, e.g., *Scyllium*, which possess no clasper glands do not rely on erection for the purpose of copulation.

The functions of the clasper gland would therefore appear to be, at any rate in the Lamna type, the same functions as those of the prostate, e.g., 1) lubrication and provision of a vehicle for spermatozoa; 2) control of erection; 3) activation of the spermatozoa; 4) providing nourishment for the spermatozoa. Erection is most marked in *Raia*, *Rhina*, and *Lamna*.

Considering Barrington's objection that the erection is due rather to the katabolite of a muscle than to glandular secretion, attention was particularly drawn in my preceding memoir to muscle of a peculiar type in connection with the clasper gland of *Raia*. In fact, we see that all the glands are well supplied with muscular coats (contrast the rectal gland of *Scyllium* to be mentioned presently). It may be, then, that a function for this specialized muscle is hereby indicated.

Turning from the physiology to the embryological aspect of these glands, I have endeavored in figure 9 to diagrammatize their development so as to show their analogy and possibly homology with the prostatic glands of mammalia. In these sketches the proctodaeum and epidermal structures are indicated by the broken lines, the urinogenital system, cloaca, etc., in plain unbroken lines, and the accessory glands (prostate, Bartolini's, Cowper's, clasper) in solid black.

Series A, nos. I to IV, shows ventral diagrammatic views indicating the origin of the clasper gland from an invagination of the side of the proctodaeum. IVa is IV in side view.

Series B elucidates the isolation of the clasper gland from the proctodaeum, by reason of the formation of its duct, or at least of its peripheral end, from a groove on the medial aspect of the clasper.

Series C. In nos. I and II two stages are shown in the development of the urinogenital system and accessory glands in human embryos. No. III delineates the hypothetical primitive condition of cloaca and proctodaeum with the areas from which evaginations lead to the formation of accessory glands dotted in.

These figures should further be compared with the diagrams of corresponding transverse sections of *Rhina* given in figure 12.

Some objection may be taken to the term 'prostatic' to include all these glands as in figure 9, C II. The human prostate is

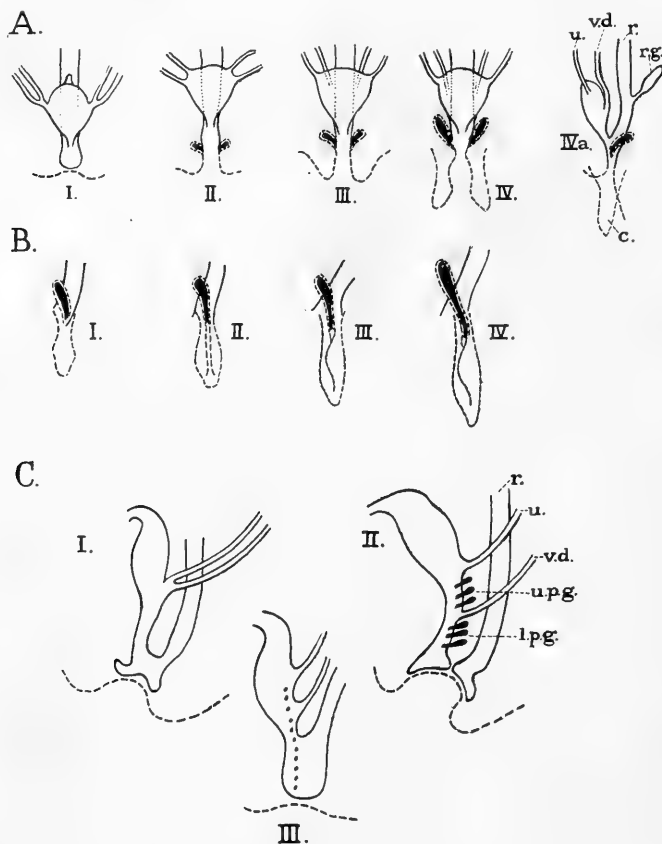


Fig. 9 For explanation see text p. 371. *r.*, rectum; *u.*, ureter; *v.d.*, vas deferens; *r.g.*, rectal gland; *c.*, claspers; *u.p.g.*, upper prostatic glands; *l.p.g.*, lower prostatic glands.

endodermal in origin and arises as evaginations of the urino-genital sinus. Cowper's and Bartolini's glands are ectodermal (epidermal) in origin and arise as invaginations of the proctodaeum. The clasper glands are also developed as invaginations of the epidermis. It is obvious, therefore, that the clasper



glands, while having a physiological function analogous to a prostate, cannot be homologous with the gland called prostate in man, but may rather be homologous<sup>3</sup> with the group, called Cowper's and Bartolini's in human anatomy, whose function is at present problematical.

Lamna is generally described as possessing open spiracles but I failed to find them, my experience agreeing with that of Günther before me. There is a deep hyomandibular cleft, but no perforation pierces the skin.

I have discovered in Lamna a large much-coiled infra-orbital gland to be described elsewhere.

#### RHINA SQUATINA

##### *The monk or angel-fish*

The specimen of Rhina upon which the following examination was conducted, taken at Plymouth in January, 1919, measured 29 in. from the tip of the snout to the extremity of the body (the cleft in the heterocercal tail), exclusive of the caudal fin. Here again the claspers are small in proportion to the size of the animal, being very slightly shorter than the pelvic fins, and measuring but 2 cm.

The monk fish was long considered a connecting link between the Selachioidei and the Batodei, though I believe this view is no longer held by morphologists. As regards the claspers, etc., it agrees with the skates rather than with the sharks.

The claspers are soft and smooth, and devoid of denticles, as in Raia. They taper to a fine point. There is no trace whatever of an apophysis. The tube leading from the siphon does not extend to the extremity of the clasper as in Raia. Instead of possessing large siphons of the type found in Scyllium and Galeus, it resembles Raia, or more closely Lamna, in having a short clasper gland, in this case 3 cm. in length (fig. 10).

Jungersen in 1898 discusses at some length this gland which he

<sup>3</sup> Possibly 'homoplastic' would be a clearer expression to indicate parallel development.

calls the 'glandular sac.' According to him, the sac is so large, standing out in relief under the skin, that he compares it with the calf of a man's leg. He does not state the size of his fish, and the circumstance that no such raised appearance of the sac was visible in my specimen may have been due to its smaller size or to the absence of much secretion. In this connection it is interesting to note that this author states that the glandular sac is full of mucus, and my investigations show that the gland of *Rhina* is simply and solely a mucous gland (fig. 8, C). It is unfortunate that the investigator, though perfectly correct in

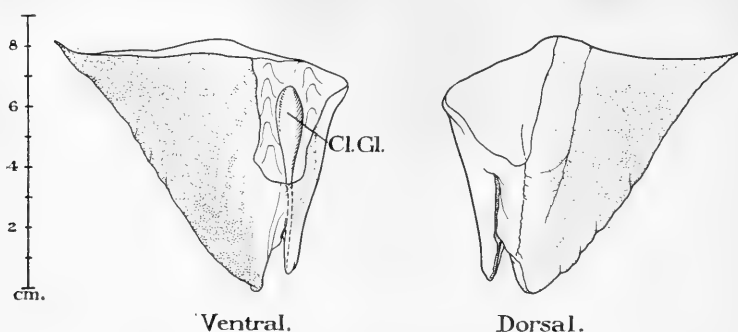


Fig. 10 *Rhina squatina*. *Cl.Gl.*, clasper gland

this instance, should have been led to generalize that clasper glands are always mucus-secreting for in its histological structure the gland of *Rhina* differs from that of *Lamna* and *Raia*. [The gland arises as epidermal patches, and is at first superficial at or near the point where the clasper adjoins the pelvic fin. More anteriorly, glandular tissue surrounds the imperfectly closed tube of the clasper and finally becomes a completely closed gland surrounding a lumen. These gradual transitions I have endeavored to diagrammatize in figure 12 from various selected transverse sections. In these sketches the supporting cartilage is black; the small circles indicate sporadic masses of blood corpuscles marking the presence of erectile tissue; the dotted portions reveal the glandular tissue. I and II are across the clasper. The cartilaginous skeletal support is a single rod, erectile tissue is present, but as yet no gland.

As we approach the body, in III, IV, and V, so as to include some portion of the pelvic fin in the section, masses of glandular tissue appear, at first sporadically around the clasper groove; finally they form a complete investment as in VI. Superficial masses of gland at first appear in III and continue till V, though only indicated for simplicity's sake in IV. The region shown in IV is given in full microscopical detail in figure 11. From IV onward other skeletal supports appear.

VI, VII, VIII, and IX give various aspects of the gland in transverse section as it narrows anteriorly, and figure 13 is a vertical longitudinal section corresponding to the region IX.

The gland itself is composed of the usual stratified epithelium, in which, quite close together, are numerous goblet-cells of truly colossal dimensions. In the superficial layers of gland there may be as few as one layer of goblet-cells, with smaller cells containing well-marked granules and similar to those of the epithelium between them (fig. 8, C). As the belt of gland increases, so do the numbers of rows of the goblet-cells (figs. 13 and 14).

These goblet-cells are very much larger than those met with in the colon, etc., of the frog and of mammals, but like them have the cytoplasm and nucleus of the cell pushed to the inner end, almost the whole cell being filled with the unstainable mucous secretion.

The subject of erectile tissue has already been touched on in *Raia* in the preceding memoir. In that species I have seen claspers in a state of erection. In figure 11 the tracts of erectile tissue are indicated by the presence of the red blood corpuscles. There is even more erectile tissue in the claspers (fig. 12, I and II), and it will be seen to lie mainly on the side away from the skeletal support. When the gland becomes closed, erectile tissue completely surrounds it (fig. 12, VI and VII). This appears to corroborate what was previously said, under the heading of *Lamna*, that erection is possibly brought about by a metabolite of the muscle surrounding the gland, as has been supposed to be the case in the human prostate. I may be permitted to repeat that erection is most marked in, if not entirely

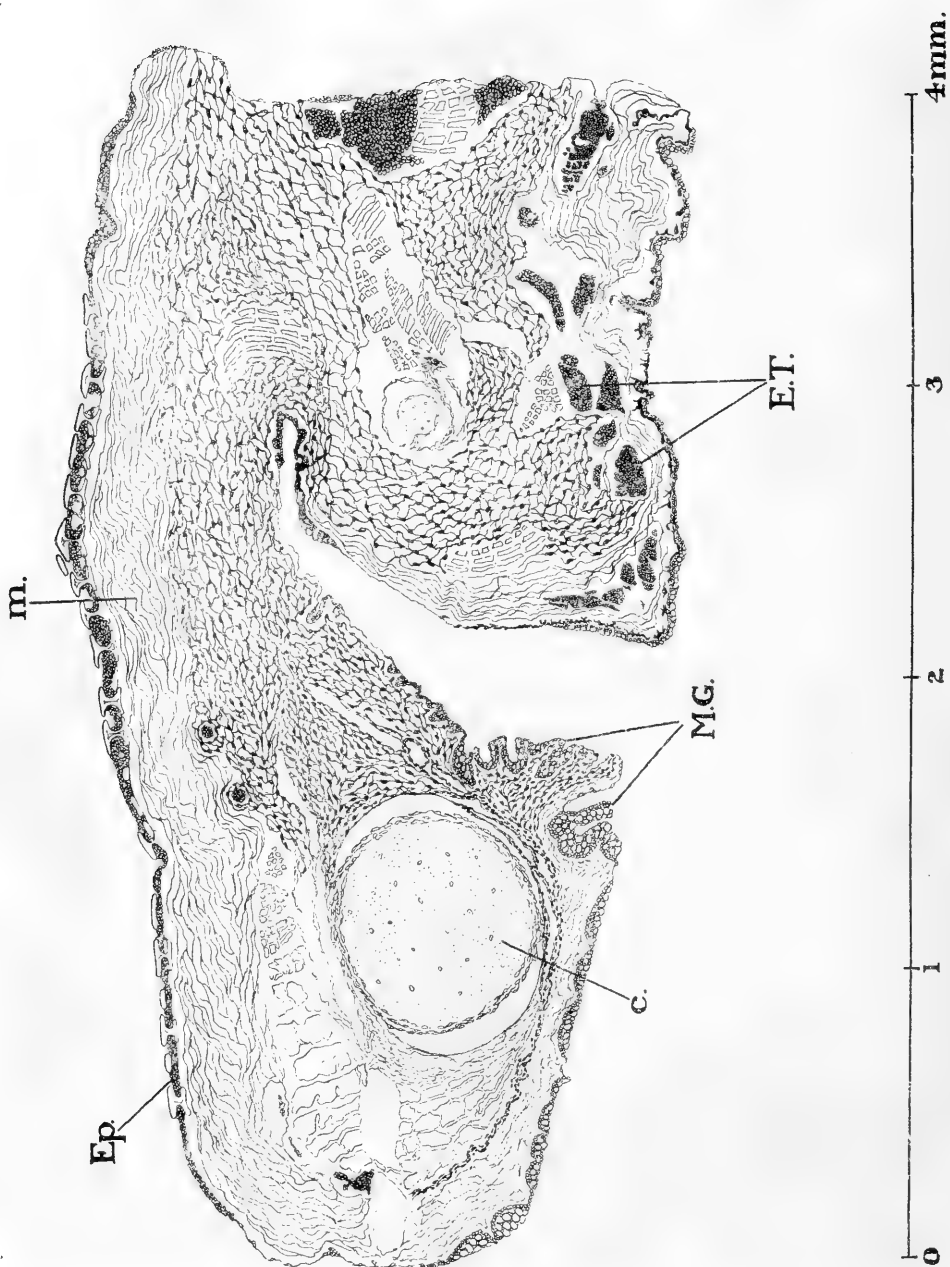


Fig. 11 *Rhina squatina*. Transverse section of the clasper and its glandular tissue near to where it joins the body wall (diagram IV in fig. 12). Alum-cochineal. *Ep.*, epidermis (note dermal denticles); *m.*, muscle; *C.*, cartilage; *MG.*, mucus gland; *E.T.*, erectile tissue with blood-vessels (note pigment cells below epidermis in this region).

confined to, the smooth claspers of *Raia*, *Lamna*, and *Rhina*, which possess a gland, however different in function the other cells of the gland may be, and is not discernible in types possessing siphons and roughly denticled claspers, such as those of *Mustelus*, and *Scyllium*.

#### THE LABIAL ORGAN

On the outer border of the upper lip of *Rhina squatina* near the commissures on either side is a small patch of tissue which

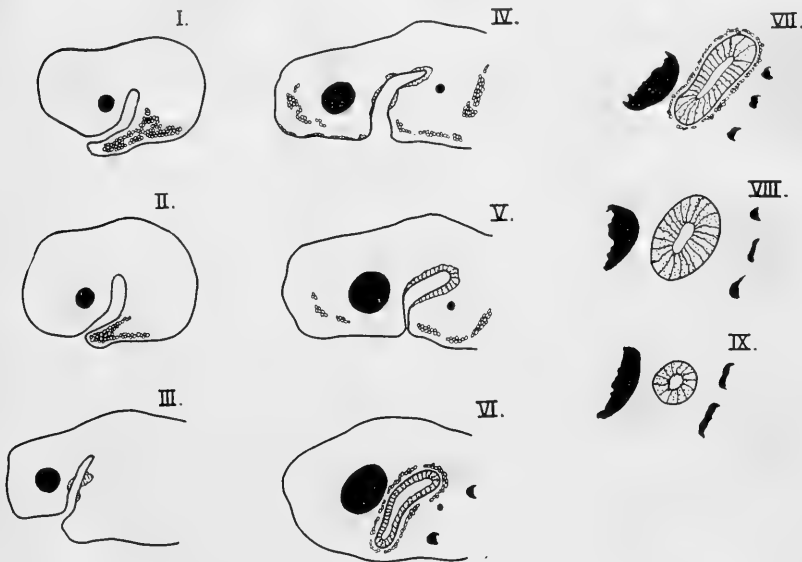


Fig. 12 *Rhina squatina*. Diagrams of transverse sections of the clasper gland to show its epidermal origin and subsequent invagination to form a closed tube.

one would conjecture from its position to be a sensitive area or organ of special sense, e.g., smelling. I was much surprised, therefore, on sectioning this so-called organ to find that it consisted of a mass of goblet-cells, and was precisely of the same

type, nature, and conformation as the clasper gland of the same animal, and that instead of being an organ of special sense it was obviously a mucus-secreting gland (fig. 14).

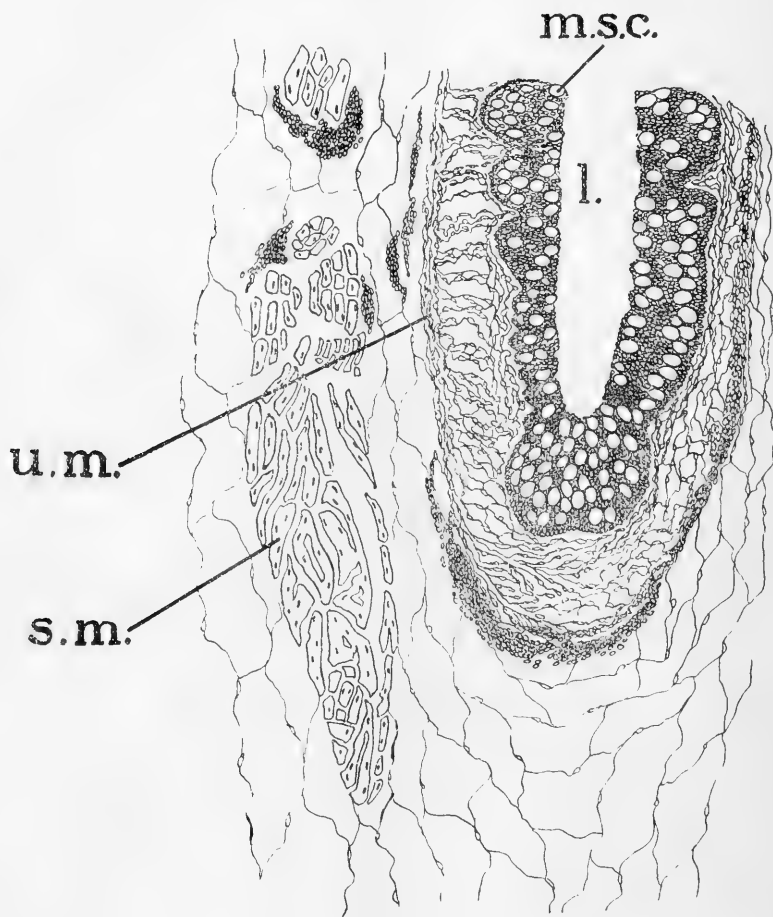


Fig. 13 *Rhina squatina*. Vertical longitudinal section of the anterior end of the clasper gland. Alum-carmine. *l.*, lumen; *m.s.c.*, mucus-secreting cells; *s.m.*, striped muscle; *u.s.*, unstriped muscle.

## ADDENDUM

## THE RECTAL GLANDS OF ELASMOBRANCHS

It is generally believed and commonly taught that the rectal gland of *Scyllium* is a mucous gland. A certain text-book makes the statement that it "is accessory to the thyroid." Accordingly, in September, 1919, I sectioned the rectal gland of *Scyll-*

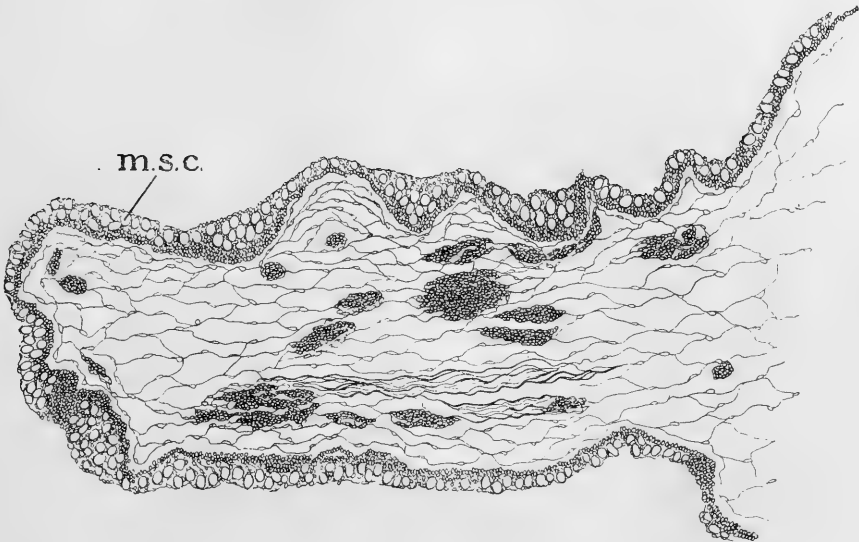


Fig. 14 *Rhina squatina*. Transverse section of the labial organ. Alum-cochineal. *m.s.c.*, mucus-secreting cells.

ium with the result that neither assumption appears to be correct (fig. 15).

The muscular coat surrounding the gland is feebly developed and the whole organ is poorly supplied with blood-vessels—a remarkable circumstance in view of the fact that the gland receives apparently the whole supply of the posterior mesenteric artery.

The glandular portion of the organ consists of a number of convoluted tubules, which, except at their entrance to the

rectum, occupy the whole diameter to the exclusion of any lumen. Their structure, appearance, and arrangement suggest that of the mammalian kidney except that there are no malpighian bodies and the blood supply is very slight. It is possible that this organ is excretory in function and may get rid of poisonous substances not eliminated by the kidneys.

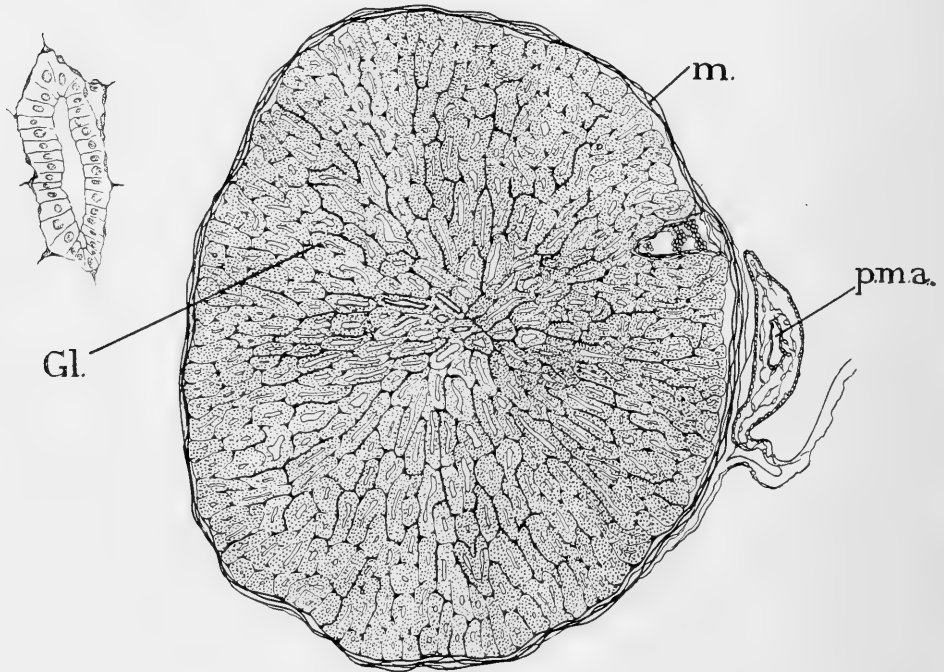


Fig. 15 *Scyllium canicula*. Transverse section of the rectal gland about its middle, with a small inset of a single tubule of the gland under a high magnification. *p.m.a.*, posterior mesenteric artery; *m.*, muscle; *Gl.*, gland.

#### ERRATUM

JOURNAL OF MORPHOLOGY, vol. 34, no. 2, September, 1920, MEMOIR I, page 256, figure 5, *longitudinal* should be *transverse*. The scale of this figure is incorrect and should be 0.1, 0.3, 0.5 mm.





Resumen por el autor, Harry H. Charlton,  
Osborn Zoological Laboratory, Yale University.

### La espermatogénesis de *Lepisma domestica*.

*Lepisma domestica*, aunque es un insecto primitivo presenta un proceso complicado durante la espermatogénesis. En la espermatogonia existen 34 cromosomas, pero en vez de encontrar 17 en el espermatocono primario, existen 18, puesto que dos de los cromosomas espermatogoniales no se unen. En la division que sigue, estos dos idiocromosomas pasan indivisos a uno de los polos, de tal modo que los espermatoconos secundarios poseen 16 y 18 cromosomas, respectivamente. Los mismos números existen en las espermátidas al separarse los idiocromosomas durante esta división.

El autor ha seguido paso a paso la historia del centrosoma y su persistencia indica que es una estructura permanente, aun cuando puede cambiar en apariencia. En la espermatogonia se presenta en forma de gránulo esférico; en el espermatocono primario exhibe forma de V, y en la figura de división del espermatocono secundario aparece en forma de bastoncito. Cada espermátida posee uno de estos centrosomas, y de él nace el filamento axial. Mediante rotación de la célula el centrosoma viene a tomar una posición terminal y más tarde forma el acrosoma, mientras que el filamento axial viene a ponerse en contacto secundario con el segmento intermedio. El desarrollo de este último órgano no es completamente claro, pero al parecer deriva del nebenkern. La porción del filamento axial situada entre el acrosoma y el segmento intermedio se transforma en la membrana ondulatoria del espermatozoide. El autor ha prestado alguna atención a las mitocondrias, los restos fusoriales, la formación del nebenkern y los cambios que experimenta.

# THE SPERMATOGENESIS OF LEPISMA DOMESTICA

HARRY H. CHARLTON

*Osborn Zoological Laboratory, Yale University*

SIX PLATES (NINETY-FIVE FIGURES)

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## INTRODUCTION

Cytologists have long found the class Insecta to be a very fertile field for investigation, but the first order and, according to many, the most primitive, namely, the Thysanura, seems to have been neglected. According to Harvey ('16), only three papers dealing in any way with the cytology of any closely

related forms have been published. The writers, Claypole ('98), on *Anurida maritima*, Lecaillon ('01), on *Orchesella villosa*, and Willem ('00), on *Podura aquatica*, simply report isolated observations which are necessarily incomplete and limited to the class Collembola.

The Lepismatoidea have therefore never been made the subject of a cytological study, and it was in the hope that a survey of this primitive form would throw some light on the present-day cytological problems that this investigation was undertaken.

The completion of the study shows that, instead of the expected simplicity, the process actually is a complicated one, differing only here and there from that already described in other forms. These differences, however, are interesting and, together with the fact that it is the first cytological work in a new class of insects, warrant its presentation.

The work was done at the Osborn Zoological Laboratory at the suggestion of Professor Petrunkevitch beginning in the fall of 1916. During 1917-19 it was practically suspended except for an occasional day or so at Columbia University. It gives me pleasure to express my thanks to Prof. E. B. Wilson for his kindness in giving me laboratory privileges at Columbia and to Prof. Frank R. Lillie for facilities accorded at the Marine Biological Laboratory, Woods Hole, Massachusetts, during the summer of 1919.

Most of all, I am indebted to Prof. Alexander Petrunkevitch, first, for suggesting the problem and later for his unfailing help and criticism.

#### MATERIAL AND METHODS

*Lepisma domestica*, commonly called the fire-brat, from its frequently observed habit of running apparently unharmed over hot stones in bakeries, belongs to the class Thysanura of the order Lepismatoidea. It is a fairly common insect in New Haven, and can be kept alive in the laboratory for a considerable period. My method has been to keep them in large glass Stender dishes without covers, since the insects cannot climb up a clean glass wall, and to provide them with a cereal such as corn flakes

to eat. For moisture I have kept shallow dishes filled with moist filter-paper in the Stenders. In spite of these precautions, in the course of a month or so the creatures begin to show a shrinkage of the abdominal region and soon die with the posterior region shrunken fully one-half.

The insects were either killed in xylol or decapitated and the testes dissected out immediately in physiological salt solution. As soon as the body cavity was opened, some fixing fluid was introduced by means of a fine pipette. This renders the tissues more opaque and makes it easier to locate the gonads which are loosely surrounded by fat, as well as to cause better fixation.

For counting chromosomes, Bouin's fluid at 38°C. proved the best, but for general fixation of the cytoplasm as well as of chromatic structures nothing equaled Flemming's strong solution. In addition, Hermann's fluid, Benda's Flemming, 10 per cent formalin, Allen's modification of Bouin, Petrunkevitch's fluid, and Kopsch were tried out and had their special uses.

The testes dissected out, fixed, washed, and dehydrated, were imbedded in paraffin and cut into sections from 3 to 12  $\mu$  thick. Sections of 7  $\mu$  thickness were found to be very satisfactory for study, and in general this was the thickness used.

The stain used generally throughout was Heidenhain's iron haematoxylin without any counterstain. In addition, various counterstains were tried, and also, in an effort to get a selective stain for mitochondria, Benda's alizarin-crystal violet method. A modification of Cajal's silver-impregnation method by Hortega ('16), especially recommended for centrosomes, was given an inadequate trial with but fair results.

I have also examined a number of splendid slides stained by the safranin-gentian-violet-orange G method, for which I am indebted to Dr. P. W. Whiting.

## OBSERVATIONS

*External sexual characters*

During the spring months of March, April, and May, the adult insects are in the best condition for study, but since it takes some time, probably a year or more, to attain sexual maturity, the early stages may be studied at any time of year in young individuals.

It is comparatively easy to recognize the sexes by their external appearance. The female (fig. 2) has a long median ovipositor extending posteriorly which is quite prominent in the living insect. There is nothing comparable to it in the male (fig. 1), for the penis which could not possibly be confused with the ovipositor is more often retracted and not in view.

*Male reproductive system*

The testes (fig. 3), of which there are three pairs on each side of the middorsal line, occupy, in mature individuals, a considerable portion of the anterior two-thirds of the abdomen. The testes lie parallel to each other, extending in a ventroposterior direction, and each is connected by a short duct with the vas deferens, which passes as a straight duct posteriorly where it enlarges to form the seminal vesicle. From the seminal vesicle a similar duct extends, which soon enlarges considerably and, after bending upon itself a couple of times, opens into the base of the penis.

*Spermatogonia*

I have made a long and careful search for primary spermatogonia in the youngest material at my disposal, which consisted of insects only 2 or 3 mm. long, but have not been able definitely to identify them. It is therefore very probable that the primary spermatogonia occur only very early in the life-history. Munson ('06) has described an apical cell which produces early spermatogonia, but this, too, if present at all, would be found in exceedingly young individuals.

One does find cells in quite large numbers at the blind end of the testis, which differ from the ordinary spermatogonia in having large homogeneous nuclei with the chromatin condensed into a single dense mass and irregular in shape (fig. 28). I believe these to be immature Sertoli or nurse cells, for later on one finds such cells, only now they are larger, more elongated, and contain two to four chromatic bodies. Those at the region of the mature spermatozoa are much paler in color and may be wrinkled and twisted upon themselves, indicating perhaps degeneration.

The spermatogonia occupy a considerable part of the blind end of a tubule of the mature insect during the winter and early spring, and can be easily recognized by their position and by the arrangement of the chromatin in the form of clumps attached to each other by linin threads and grouped around the periphery of the nucleus. This arrangement (fig. 4 is a surface view) is the most common and probably represents a resting condition. Although I have not been able to count these clumps of chromatin, the number is easily seen to be more than the haploid, and each one probably represents a spermatogonial chromosome.

The nucleus of the early spermatogonium is quite large and almost equal in size to the nucleus of the growth period. The two or three spermatogonial divisions reduce the nuclear and cell size by apparently not allowing time for growth between divisions (figs. 8 to 11). In prophase the chromosomes are long and bent upon themselves and irregularly scattered throughout the nucleus; later they are drawn into the metaphase plate as shown in figure 5.

It is only in the larger and therefore the earlier spermatogonia that good counts of the chromosomes can be made. Figure 6 shows thirty-four chromosomes in a very clear metaphase plate. The chromosomes are of the curved-rod type, differing considerably in size, but close observation fails to show any chromosome or group of chromosomes behaving in any way differently from its neighbors.

In the telophase of the spermatogonial division the chromatin becomes granular and forms a more or less eccentric ring around

the nuclear wall (figs. 11 and 16). At a little later stage one commonly finds an irregular clump of chromatic material representing apparently two spermatogonial chromosomes lying against the nuclear membrane and retaining the haematoxylin stain (figs. 13, 14, and 15). For these chromosomes I shall use the term *idiochromosomes*, the name given by Wilson ('05) and meaning 'peculiar or distinctive chromosomes.'

In some presumably young cysts the spermatogonial cells are arranged in the form of a rosette, their median ends tapering toward a faintly marked open center and showing an archoplasmic mass or sphere (figs. 4 and 18). Hegner ('14), Meves ('97), and Shaffer ('17), as well as others, have described similar structures and considered them spindle remains. In some cases they figure them as extending from cell to cell. While this is true immediately after division when the spindle remains are very definite (fig. 7), it is not possible later to see any continuation or connection with similar bodies in adjacent cells.

In the spermatogonial region isolated cells are occasionally seen in division, but, strangely enough, the chromosomes are paired and look somewhat like tetrads (fig. 32). The cells themselves are much larger than the spermatogonia and contain but little cytoplasmic staining material in the form of a flaky mass at either end of the cell in which a dark-staining granule may be seen. If these represent division in the Sertoli cells, they are a very rare occurrence. In the older Sertoli cells I have occasionally seen evidence indicating division by amitosis.

### *The growth period*

The stages of the growth period correspond fairly closely with the stages described by Wilson ('12). After the telophase, chromosomes of the last spermatogonial division break up and form a granular ring just inside the nuclear wall, the chromatin arranges itself as previously described in the form of clumps located on the nuclear membrane (Wilson's stage b, similar to fig. 13). In heavily destained material two of these are closely related, one of them being flattened against the nuclear mem-



brane and retaining the dark stain of the haematoxylin (fig. 15). In addition to the idiochromosomes, a similarly staining, small, spherical granule appears (fig. 13). The chromatin clumps now become granular and form an eccentric circle against the nuclear membrane, leaving an open center very much like the condition following the last division. The homogeneous granular border is at first deeply stained, but later loses its affinity for the haematoxylin and appears pale in color (figs. 11 and 16).

The idiochromosomes also seem to break up into unequal spherical bodies, three to eight in number, six being the more common number (fig. 16). In the clear central region the remains of the preceding spindle are quite apparent. Following this stage we have the reappearance of the idiochromosomes (fig. 17), and after that the entire nucleus appears granular, the central clear area disappearing and the two idiochromosomes stand out clearly (fig. 21).

It has not been possible to see anything like an unraveling stage as described by Wilson ('12) for stage c; the granular condition being directly followed by delicate threads (Wilson, stage d, fig. 22) which seem to push out and distort or break the nuclear wall. This is soon followed by the synizesis or contraction stage. Here the threads are drawn closely together and are located more to one side of the nucleus, the plasmosome and idiochromosome thread often remain outside of the contracted mass, as shown in figure 23.

Popoff ('08), Gates ('08), and Whiting ('17) look upon this as due to a rapid absorption of water by the nucleus; in other words, an osmotic effect; however, it has often been considered an artifact. Although at this stage of the growth period the spireme threads stain very intensely, making it difficult to trace the individual threads, it would look as though the filaments became arranged in the form of loops polarized with their free ends near the plasmosome and idiochromosome threads. Later on when the threads have thickened, this bouquet stage is much more clearly seen (fig. 25).

It has not been possible to see a side-by-side union of the spireme threads, the synapsis of Moore ('95), but the number

of filaments certainly is reduced and each one becomes much thicker. The threads now loosen up and occupy practically all the cell, the space between the nuclear membrane and the cell wall being quite small (fig. 24, Wilson ('12), stage f). I have not been able to find the longitudinal splitting of the thread—a process which Wilson ('12) describes as taking place.

There follows a period when it is hard to distinguish the threads as such (fig. 29, Wilson ('12), stage g). Wilson calls it a net-like arrangement. The actual breaking of the threads or pachytene stage is not well exemplified in *Lepisma*, but stage g is soon followed by the clumping of the chromatin into masses irregular in shape and joined together by linin threads (fig. 30). By a further condensation of these masses we get the prochromosomes. The formation of tetrads showing the quadrivalent condition of the autochromosomes is never apparent, neither is there any split indicating a parasynapsis.

The idiochromosomes retain their form and staining reaction until the formation of the delicate filaments (stage d), when they break up and form threads which are darker in color than the other threads, and one may be seen in close relation to a small plasmosome (fig. 35 a). The idiochromosome threads are at first very long and may extend across the entire width of the cell. They appear somewhat beaded, just as is the case with the threads of the autochromosomes.

During the later periods the threads show an end-to-end apposition, being joined by very fine linin fibers (fig. 35 m). The threads now become shorter and thicker assuming the U shape followed by the definite formation of loops with the plasmosome between them (figs. 20 and 35 j). Figures 35 i and h would seem to indicate that the limbs of the loop come together and become still more compact to form clumps lying against the nuclear membrane with the plasmosome still lying between them. For a considerable time the idiochromosome threads show a very clear inequality in that the thread nearest to the plasmosome is the longer (fig. 35 k).

A second small plasmosome may be formed and lies to one side with no attachment to either idiochromosome thread (fig.

35 e and f). Later I believe it fuses with the first, for the latter is seen to increase considerably in size and to show at times a double nature (fig. 35 g and k).

A third body similar in shape and staining reaction to that seen in the spermatogonia becomes quite prominent at this time (figs. 29 and 30), due to a slight increase in size and to the appearance of a clear transparent area encircling it. Painter ('14) describes in spiders similar small dark-staining spherical bodies, which he calls planosomes and which first make their appearance in the late spireme stage and which he was able to trace through the succeeding divisions. The planosomes, according to him, have spindle fibers, and would therefore be comparable to chromosomes, although as a rule they do not divide, but linger near the middle of the spindle and later go to one side.

From his description and figures, this body is the same as the one found in *Lepisma domestica*, only I find it first in the resting stages of the spermatogonia, and have not been able to follow it beyond the prophase stage of the first maturation division.

#### *The first spermatocyte*

With the condensation of the chromatin segments into the prochromosomes, the nuclear membrane breaks down and two chromosomes located near the periphery are seen joined together by a more or less ribbon-like connection, forming a V-shaped structure. Within or near the arms of the V the plasmosome may be found (figs. 36 and 37). With the exception of the prophase figures in which the idiochromosomes stain more deeply, there is no essential difference in the staining reaction of the idiochromosomes and the autochromosomes; but to make the behavior of the idiochromosomes plain throughout the different stages of the first maturation division, they have been drawn in black, while only the outlines of the autochromosomes are shown (figs. 36, 37, 41, 42, 43, 44, and 45).

The chromosomes arrange themselves on the spindle and in the metaphase plate (figs. 38, 39, and 40), the sex or idiochromosomes are still connected and one pair of the chromosomes is a

little further beyond the metaphase plate, so that in plate view one pair of chromosomes can be seen to be at a different level (figs. 39 and 40). The side view shows how one limb of the V extends farther than the other.

The metaphase plate (fig. 38), in which the idiochromosomes are located in the center and surrounded by a ring of chromosomes, reminds one of the arrangement in some Hemiptera.

There is little change in the position of these joined chromosomes in the anaphase (fig. 41), except for a shortening of the connecting thread and possibly a slight movement of the whole toward the distal pole. Figures 42, 43, and 46 picture the telophase arrangement, the idiochromosomes going undivided to one pole.

There are sixteen chromosomes plus the two idiochromosomes, or eighteen in all, in the first spermatocyte division. Side views have not been counted, owing to the great overlapping of the chromosomes. The plasmosome may be identified during the late prophase (fig. 37), but not definitely after the actual spindle formation. Bodies which are plainly not chromosomes are often seen in relation to the spindle, as the two equal bodies in figure 40, but whether these represent the divided plasmosome or are mitochondrial is not conclusive.

#### *Resting stage of second spermatocyte*

In the telophase of the first or early prophase of the second spermatocyte (fig. 46), the chromosomes are breaking up. Some appear unchanged, while others have swollen to a spherical shape and stain more diffusely. It is not possible to identify the idiochromosomes at this time, but a little later, when the resting nuclear stage is reached, the double nature of the idiochromosomes is quite apparent as the nucleolus in one of the now divided cells (figs. 47, 48, 50, and 51). It is not possible to confuse these resting second spermatocytes with the early spermatids, because both nuclear and cell size is much larger. The relative sizes of first and second spermatocytes and spermatids are shown in figure 33, which was diagrammed from measurements of the length

and breadth of ten representative cells of each kind, and the average diameter of each cell and of the ten cells taken.

In the second place, the chromatic nucleolus is distinctly double (fig. 47), while in the spermatid it is single and smaller (figs. 64, 66, and 68).

During the growth and division period, spindle remains stand out quite clearly as one, more usually as two vesicles, formed probably from the central fibers and showing a granular condensation in their interior (figs. 25 and 46).

The formation of the resting stage and the subsequent prophase is a rapid one, as I have observed resting nuclei, prophase, and dividing second spermatocytes in the same cyst. Figures 50 and 51, resting and prophase stages, respectively, are from a slide not particularly well fixed, as the cells are somewhat swollen, but figure 51 is interesting in that it shows the formation of spindle fibers before the nuclear wall has broken down and in figure 50 the idiochromosomes still show their double structure. The resting nucleus, at first granular, breaks up into faintly staining irregular or fantastically shaped entities without any visible unraveling stage and condense quickly into the prochromosomes (figs. 49 and 50).

#### *The second maturation division*

With the formation of the spindle for the second maturation division, two types of metaphase plates are seen: one (fig. 53) with eighteen chromosomes and another (fig. 56) with sixteen. In the latter case I have one perfect anaphase (fig. 59), in which both plates can be counted and both show sixteen chromosomes.

It appears that the idiochromosomes are now equal in size and no longer show a connecting thread. In the first maturation division the idiochromosomes were distinctly unequal, but each tapered into a thread connecting it with the other. This thread often seemed ribbon-like, granular, and taking the iron haematoxylin stain like the chromosomes.

It seems to attain its maximum length at the metaphase of the first maturation division and to shorten a great deal by the

time the telophase is reached, and it would appear as though this thread were fused with the smaller idiochromosome so that they both appear equal in the metaphase of the second maturation division. Another factor in favor of this hypothesis is that the chromatic nucleolus of the resting stage shows a double structure with hardly any inequality.

In the early anaphase (fig. 55) all the chromosomes show a longitudinal split near their centers, except two which represent, I believe, the divided idiochromosomes. The anaphase often shows the chromosomes arranged in the form of a ring (fig. 62). In figure 63 the chromosomes are at the poles and are beginning to form a nuclear membrane, but no change has taken place in the centrosomes. Figure 60, a late telophase, shows that one chromosome differs from the rest in being elliptical, while the others are V- or U-shaped and slender. A still later telophase is figured in figure 61, the chromatin now being massed at the poles. Two types of spermatids are formed, those with sixteen and eighteen chromosomes, respectively.

*The centrosome in the spermatogonial and maturation divisions*

In the archoplasmic mass or sphere representing the remains of the previous spindle one may occasionally see two dark granules (fig. 18), which I take to be the divided centrosomes. In the division figures of the spermatogonia centrosomes are difficult of demonstration, but in a few slides I can make them out as definite single granules at the poles of the spindle (fig. 12). I have never seen astral rays or anything comparable to a centrosphere at the time of division, but during the resting stages the centrosome is found in a granular sphere.

From the division figure of the last spermatogonial mitosis until shortly before the synaptic or contraction stage, the centrosome has not been traced, and when it does appear a considerable metamorphosis has taken place. At about the time when the fine spireme threads are being changed into loops, a granular mass can be made out at one end of the cell, and in this mass appear two short, stubby rods lying parallel to each other.

Later (figs. 20 and 26) the rods lengthen and show small granules at their ends. At first the two rods form an angle of  $180^\circ$ , but this angle is later decreased to  $90^\circ$  or less. Each rod now divides, but the halves remain attached by their granule ends, forming a pair of V-shaped centrosomes, each V representing a divided centrosome. This whole process is a rapid one, for all stages as well as the separation of the V's for some distance may be seen in cells which show little change otherwise. The migration is about completed and the V's nearly at the poles by the time the prophase condition is reached (fig. 30). During the succeeding division the apex of the V is directed toward the chromosomes, while its limbs touch the surface of the cell. The V may open considerably, nearly to a straight angle, so that a large part of the outer surface of the rods is in contact with the cell wall. The cells may also show a slight depression at the poles (fig. 39).

The spindle fibers all lead to the centrosome region, but an actual attachment of the fibers to the centrosomes, while taken for granted, does not show clearly in sections.

This V arrangement can be identified up to a late telophase of the primary spermatocyte, but I have not traced it through the resting stage of the second spermatocyte. Each second spermatocyte would receive one V, but when the rods reappear in the division figure they are divided, a single rod at either pole lying against the inner surface of the cell wall and oriented parallel to each other, but at a slight angle with the cell axis. The division or separation of the V's as well as their migration to opposite poles must take place during the resting period.

The centrosome rod can be traced through every succeeding stage to the early spermatid, where it may be seen lying free in the cytoplasm (fig. 65). In exceptional cases, as in figure 54, the rods have granules at their ends, or we may find a number of granules or fragments and no rod, as in figure 58.

#### *The spermatid*

The young spermatid cell is considerably smaller than the resting stage of the second spermatocyte. The chromosomes clump together, form a nuclear membrane, and quickly break

up. The nucleus appears round in polar view, but oval if looked at from the side. Later the nucleus becomes spherical, the chromatin appearing finely granular and congregated at the boundaries of the nucleus leaving an open center (fig. 66). One-half the cells show an idiochromosome nucleolus which usually presents a spherical part extending into the nuclear cavity and a flattened area against the inner surface of the nuclear membrane, while the other cells do not possess an idiochromosome nucleolus.

The methods of fixation and staining have a great deal to do with the structures observed in the spermatid. When strong Flemming is used for fixing followed by Heidenhain's iron haematoxylin, the cytoplasm of the early spermatid contains such a mass of intensely staining material that the nuclear membrane is made out only with difficulty. The same stain after Bouin's fluid brings out the nucleus and centrosomes, but not the cell inclusions.

At the very first, the cytoplasmic structures are somewhat loosely aggregated around the nucleus, but particularly between the nucleus and the last division plane. The centrosome can easily be followed from the telophase; located at first on the cell wall of the dividing second spermatocyte, it later moves inward, occupying the space between the cell wall and the nucleus (fig. 65).

As it moves around to get between the nebenkern and the nucleus, it turns  $90^\circ$  and comes to lie with one end on the nuclear membrane and the other against or near the cell wall (figs. 66 and 67). The rod-shaped centrosome now frequently shows a granule or enlargement at the nuclear end.

The nebenkern has meanwhile formed a broad ring of densely staining granular material in the center of which spindle remains of the last division appear and on either side two spherical bodies become visible (fig. 69), exactly like those seen in the two maturation divisions, and undoubtedly represent old spindles. In cross-section they appear as rings with their boundaries staining in varying degrees, often looking like crescents, and may possess a darker staining center. Looked at from the side, they take the form of rods with faintly stained material between them.



When the centrosome is in contact with the nuclear wall, one usually sees a granule at its inner end (fig. 66), and later a granule similar in size located near it on the membrane (fig. 67). This suggests the breaking away or division of the granule at the base of the centrosome.

The centrosome may now change its position, being found in the region of the nebenkern or even at the opposite side, and shortly the delicate axial filament is seen pushing from the cell and occasionally carrying a small clump of cytoplasm with it, very much as has been described by Buder ('15) in the Lepidoptera and called by him 'plasmaklumpchen.'

The single granule arising from the centrosome increases considerably in size and divides, giving rise to two granules which move apart and come to lie against the nuclear membrane and closely applied to it (figs. 65, 70, 71, 72, and 73). About this time or a little later a somewhat larger, round body condenses out of the nebenkern ring, as shown in figure 72.

Outside of the breaking up of the idiochromosomes and a slight tendency to become pale and homogeneous, the nucleus remains the same during the above changes in the cytoplasmic inclusions. In the stage which follows, the delicate axial filament is quite obvious and its outgrowth from the distal end of the rod centrosome is very clear. The rod has swung so that now it is in contact with the nucleus throughout its entire length and the thread is seen traversing the space between nucleus and cell wall (fig. 74). The nucleus contains numerous dark-staining granules. The spindle remains are prominent, their borders have increased in thickness, and now appear as irregular-shaped thick-walled vesicles. The nebenkern ring, cleared of the spindle remains and of the various granules as well as of several aggregations of granular mitochondria, now rounds itself up into an oval-shaped dense mass which later becomes round (fig. 76). No structure is at first apparent except a heavily stained granular body, but one soon sees a vacuolization of its border and we get the rosette nebenkern of many writers (fig. 74).

Of the three granules already mentioned, those arising from the centrosome have either disappeared or have become so

closely adherent to the nuclear membrane as to seem a part of it, while on the other hand the other body appears slightly larger (figs. 77 and 78).

The nucleus continues to stain darker, due to the enlargement of the chromatin granules, and these may become joined to each other and give the appearance of short threads (fig. 75). The vacuolization of the nebenkern continues at the expense of the central body which becomes smaller. The walls of the peripheral vacuoles break down, the spaces becoming larger and larger, until there is but one vacuole, which may exceed even the nucleus in size, with a small heavily staining central part (figs. 80 and 81).

From this period on, the axial filament is in close relation to the central body of the nebenkern, which in well-fixed material is now seen to be made up of a spireme-like thread. I have been able to follow it throughout the greater part of its course and I feel almost certain that it is a single continuous thread (fig. 80). The cell now begins to lengthen somewhat and the central part of the nucleus to stain heavily, the chromatin moving toward the nuclear center, leaving a clear transparent border (figs. 78 to 83).

Unless one is fortunate with his fixation and staining, the central part of the nebenkern shows no structure, but appears as a glassy elliptical body suspended in the single large vacuole by means of the axial thread, but it can be seen very clearly that the tail filament never enters the central body, but comes to lie against it. The vacuole membrane lengthens out as it increases in size, while the central thread-like structure breaks up into several large and many small vesicles (figs. 81 and 82). There are also mitochondria-like structures located between the nebenkern and the nucleus as well as some distal to the nebenkern.

The nebenkern membrane forms apparently the sheath of the axial thread, some cytoplasm forming clumps around the distal part of the thread, but the vesicles in large numbers fill the spaces between the spermatozoa as they increase in length. The middle-piece anlage enlarges, and by a turning of the nucleus, the axial filament comes to lie against it (figs. 85, 86, 88, and 90).

The body then flattens out against the nucleus and later elongates slightly (figs. 85 and 90). The nucleus lengthens, and as it does, the axial filament between the middlepiece and the centrosome does likewise. The centrosome, however, is now at the apex of the nucleus and will hereafter be considered as the acrosome.

At the time when the nebenkern membrane and its vesicles have completely broken up and are only apparent as end products ensheathing the elongated tails or located between the filaments, the nucleus is still spherical, compact, and does not take the haematoxylin stain very well (fig. 82). It still has a clear area about it and some mitochondrial material about the middle-piece anlage, which is a quite prominent body located usually on the opposite side of the nucleus from the acrosome. The axial filament arising from the acrosome at the apex of the nucleus is bent backward and passes near the middle-piece anlage.

The further changes are the loss of the clear ring about the nuclear chromatin by the spreading out of the chromatic material. The nucleus shows better staining qualities. The axial filament comes to lie nearer to the middle-piece and the latter may sometimes show one or more bubbles or vesicles, which are possibly mitochondrial, in relation to it (figs. 84, 85, and 86).

The nucleus now begins slowly to elongate and seems sometimes to pull away from the acrosome, so that part of the latter body may project beyond the nucleus. The nucleus continues to lengthen, the chromatin to appear paler in color. The middle-piece enlarges and elongates (figs. 88, 89, 90, and 91). The axial filament strand between the acrosome and the middle-piece remains applied against the nucleus and sometimes may show one or several splits in the thread, leaving an elliptical opening. In material stained for mitochondria, a cloud of granules seems to gather about the thread (fig. 87). Although it cannot be traced directly, I am of the opinion that this axial filament, which is loosely applied to the outer nuclear surface, becomes the undulating membrane of the mature spermatozoon.

Thompson ('17, p. 267) suggests the formation of the undulating membrane from a free flagellum in the Trypanosomes, as follows: "It is a plausible assumption to suppose that, as the flagellum waves about it comes to lie near and parallel to the body of the cell, and that the frill or undulating membrane is formed by the clear fluid protoplasm of the surface layer springing up in a film to run up and along the flagellum, just as a soap-film would be formed in similar circumstances." Of course the axial filament in this case is located between the nucleus and the outer cell wall, but it seems a reasonable hypothesis to think of the axial filament as having become loose from the nucleus and as able to draw out the thin layer of cytoplasm some little distance from the nucleus forming the undulating membrane.

The nucleus and middle-piece now become drawn out to considerable length, the acrosome decreases in size and we see a slight projection of the nucleus extending beyond the acrosome. The elongated nucleus stains darker and darker until no structure can be made out. From this point until the mature spermatozoa are reached I have not been able to make observations (figs. 93 and 94).

#### *The spermatozoa*

A study of the mature spermatozoa has been made by teasing the contents of the seminal vesicle in a minute quantity of physiological salt solution and either studying them alive in the solution or fixing the teased material in osmic acid fumes, hot corrosive sublimate, Bouin or strong Flemming, and staining.

The unstained living contents make an interesting study when examined by means of the dark-field microscope. In addition to the spermatozoa with their waves of movement extending from anterior to posterior end of the undulation membrane, there are a large number of small elliptical bodies performing active brownian movement. I thought at first that these bodies were the true spermatozoa and the others giant spermatozoa, but further study convinced me that this was not the case, for no tails could be found upon the small bodies, they stained only by plasma stains, and furthermore no stages in their development could be made out.

Occasionally the lumen of the vas deferens is partially filled with granules differing in size, and as the cells lining the vas deferens may show similar granules in their cytoplasm, I have considered the granular material of the lumen to be secretion products of the cells. Although the bodies present in the seminal vesicle are a little longer than broad and show a little difference in their size relations, yet I think they represent the secretion found in the vas deferens. Munson ('06) considers the epithelial cells of the vas deferens of the butterfly *Papilio* to have a secretory function, but unfortunately he does not figure or describe the process.

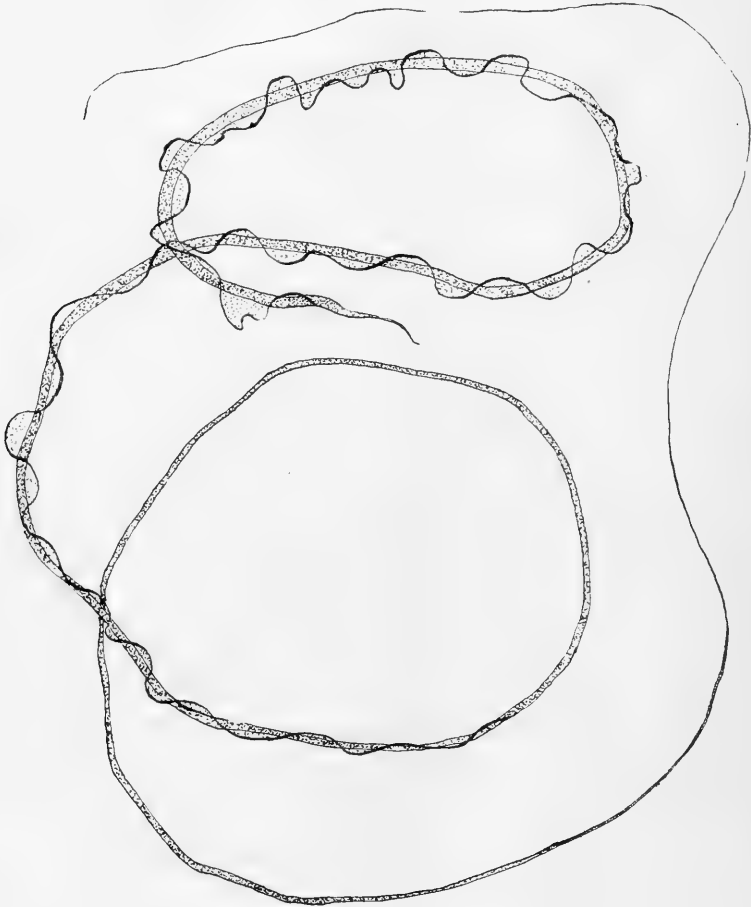
An added fact of interest is that the bodies are transmitted during copulation and are found in the seminal receptaculum of the female, which suggests that they have some function yet unknown to us.

To ascertain their nature, smears of the seminal vesicle were made, fixed in hot corrosive sublimate, and stained in orcein, safranin and orange G, Delafield's haematoxylin and orange G, safranin and malachite green, safranin and bleu de Lyon, Delafield's haematoxylin and erythrosin. In every case it was found that the granules took only the cytoplasmic stains. In order to test the possibility of the granules' being of a fatty nature, fresh smears were stained in sudan III, but the result was negative. Smears treated with ether showed also no effect of the latter on the granules.

When the spermatozoa are examined in the fixed and stained condition (text fig. A and fig. 95), one finds a long chromatin staining thread ending in a transparent fine point, the acrosome having disappeared, and extending from near the apex a conspicuous undulating membrane. The free edge seems formed of a little denser material and represents in all probability the proximal part of the axial filament, i.e., that part between the acrosome and the middle-piece.

It is almost impossible to see just where this membrane leaves off distally as it gets narrower gradually, but I should say that about the anterior two-thirds of the spermatozoon is provided with the membrane. It is not possible to find any trace of the

middle-piece or to see where the nucleus leaves off and the tail filament begins, as the latter structure becomes finer and more transparent until it is almost impossible to see where it ends.



Text fig. A Mature spermatozoon of *Lepisma domestica* arranged from successive camera-lucida drawings.  $\times 2800$ .

I have noted a tendency for the tails of the living spermatozoa to stick together. The spermatozoa are very long, measuring from 400 to 660 $\mu$ .

*Mitochondria*

Mitochondrial structures are present in the spermatogonia, but in very small numbers, and it is difficult to make them out. At the beginning of the growth period they appear clearly as a dark-staining crescent-shaped mass usually located at one end of the cell. This mass soon breaks up and forms some six to eight bodies differing from each other in shape and size (fig. 21), but retaining an almost constant number. These bodies take the stain intensely and appear as the most prominent structures during the entire growth period. As the cell increases in size, the mitochondria becomes so conspicuous, even by ordinary iron-haematoxylin staining, that the cytoplasm seems like a dark border about the nucleus, and in this dark-staining mitochondrial matrix the clumps stand out clearly.

During the first maturation division the ring of granular mitochondria encircles the entire spindle, while the larger bodies are scattered about the cytoplasm and are located near but not on the spindle. The mitochondrial material seems to divide equally, half the granular material as well as four clumps going to each cell (fig. 39). In the resting stage of the second spermatocyte the mitochondria forms a narrow dark-staining ring about the nucleus, but the clumps are no longer apparent (figs. 49, 50, and 51). In figure 54 the granular mass is seen arranged about the spindle of the second division, while figure 61 indicates how they gather about the chromatin at the poles of the young spermatids. In the description of the spermatids the further history of the mitochondria has already been given.

## DISCUSSION

*The resting stage*

While in many animals no resting nucleus is formed following the first maturation division, the chromosomes of the telophase being quickly transformed into the prophases of the second division, there are quite a number of exceptions reported in the literature.

Murray ('98) finds a well-marked resting nucleus in the Pulmonates, *Helix* and *Arion*. McGill ('04) found it to happen occasionally in the dragon-flies, and Painter ('14) describes it as occurring in the spiders with the accessory chromosome persisting as a nucleolus. Kingsbury ('01) finds in the salamander *Desmognathus fusca*, that a nuclear membrane is formed following the first maturation division, but that the chromosomes never lose their individuality.

In *Lepisma domestica* the chromosomes, with the exception of the idiochromosomes, entirely break up and a nuclear membrane is formed. While it is undoubtedly of short duration, still the outward individuality of the autochromosomes is lost and the second division is preceded by their reformation.

#### *The idiochromosomes*

Wilson ('09) divides the sexual differences of the chromosomes into five and possibly seven types. *Lepisma domestica* falls in line with his type IV in which "the male has a pair of idiochromosomes, half the spermatozoa receiving both and hence two more than the other half."

Only one form has been found which has this arrangement, the coreid species *Syromastes marginalis* L. This form was first described by Gross ('04) and again by Wilson ('09). The accessory chromosome arises by a synapsis of two spermatogonial chromosomes which divide equationally in the first spermatocyte, but fail to divide in the second.

*Lepisma domestica* differs in that the two spermatogonial chromosomes do not fuse, but remain separate and joined by a stout thread. They pass undivided to one pole in the first spermatocyte division, but separate in the second. Wilson's prediction that the female of *Syromastes* would have two more chromosomes than the male, he afterward found to be the case. Reasoning in a similar manner, *Lepisma domestica* females should have thirty-six chromosomes, but unfortunately I have been unable to make any chromosome counts so far in the female.



*Synapsis and reduction*

It has not been possible in the ordinary chromosomes or auto-chromosomes to see whether there is either a side-by-side union of the spireme threads, a parasynapsis, or an end-to-end conjugation, a telosynapsis. It is clear, however, that the spireme threads in postsynaptic stages are much thicker and are present in fewer numbers. Whether they are half the leptotene number or not could not be made out.

In the case of the idiochromosomes the conclusions are clearer. Each idiochromosome breaks up into a spireme thread and the two threads eventually unite end to end, one of them being attached to a large plasmosome. From these threads two chromosomes are formed by the condensation of the chromatin, but they still remain united by a thread which is probably linin in nature and along which, when the thread lengthens, the chromatin is drawn out.

Synapsis, or a side-by-side conjugation, if it takes place at all, does so following the telophase of the first maturation division. That the idiochromosomes do come into a very close relation is shown by the longitudinal split apparent in the idichromosome nucleolus of the resting nuclei of the second spermatocyte (figs. 47 and 50).

If, as is generally conceded, the spermatogonial chromosomes represent two groups, one of maternal and the other of paternal chromosomes, and the homologous pairs conjugate at synapsis then each of the idiochromosomes represents one spermatogonial chromosome. A further proof of this is that the thirty-four spermatogonial chromosomes, judged from their size, are all of the same valence, i.e., bivalent. After synapsis the autochromosomes are quadrivalent, but definite four-part tetrads are not apparent during the prophase, and at the metaphase the chromosomes are dumb-bell-shaped, the longitudinal pairing leaving no trace. However, in one cell I have found the idiochromosomes at the time of anaphase showing a bivalent construction (fig. 45).

The first maturation division separates the dumb-bell-shaped chromosomes transversely (fig. 39), and probably represents a reduction division, as the idiochromosomes go to one pole undivided. The second division of the autochromosomes is clearly a longitudinal one (fig. 55), while the idiochromosomes separate transversely, so that it would seem that this represents an equational division of the autochromosomes and the separation of the idiochromosomes one from the other.

### *The centrosome*

The single- or double-rod type of centrosome has been described in a variety of forms. Meves ('98) and Buder ('15) have described them in the Lepidoptera, and Sewertzoff (Meves, '00) in Orthoptera, and Korff ('01) in the Coleoptera. In plants Von Mottier ('98) found them in the tetraspore mother cell of *Dictyota dichotoma*. Korff also found them in the sperm cells of the domestic hen and duck, while Hortege ('16) figures them for the ganglion cells and brain of man.

Von Mottier does not consider them homogeneous, but to arise from small granules. In the beetles, Korff shows them to be very like those found in *Lepisma domestica*, but he has not reported any granule in relation to the rods at any time. He finds the limbs of the V separating in the late telophase and appearing parallel to the polar axis in the spindle. In *Lepisma domestica* the centrosomes are always oblique to the axis, but parallel to each other.

We have seen the centrosome first as one or two small granules, then as double rods with or without end granules, and still later as single rods which only occasionally show a granule. In very rare cases, instead of single rods, the centrosome consists of several granules in the polar position. From these observations the form of the centrosome would seem to be a variable quantity. It is interesting in this regard that Korff ('01) was only able to see the V-shaped centrosome in the sperm cells of the drake and rooster, while all the other cells of the body showed centrosomes consisting of single granules.

*The acrosome*

Although a number of the older writers, notably Platner ('89), Niessing ('96), Field ('95), and Moore (94'), have described the acrosome as arising from the centrosome, Wilson ('06) comes to the conclusion that the work of Henking ('91), Wilcox ('96), and Paulmier ('99) show conclusively that in the insects the acrosome is derived from the nebenkern.

That this is not the case in *Lepisma domestica* can be easily proved, for in every stage from the telophase of the second division until the oldest transformation stage in which it was possible to identify structures, the centrosome rod and its change into the acrosome can be followed.

It might perhaps be argued that the granule is really the centrosome and the rod only a product of the centrosome, formed in somewhat the same manner as the 'battonet' or rodlet in the spermatid of the Pribilof fur seal. As described by Oliver ('13), it arises as a prolongation from the anterior centrosome. In any event, the acrosome owes its origin either directly or indirectly to the centrosome.

Goldsmith ('19) describes in the tiger-beetle a condition, which in view of my own work on the acrosome and middle-piece, is very suggestive. He figures an extra nuclear plate or middle-piece which is formed at the point of junction of the axial filament and the nucleus. It contains several chromatin-staining bodies to which the axial filament is attached. These chromatin bodies move to one side and then toward the anterior end of the nucleus, the filament coming at last to lie against the elongated mitochondrial body (nebenkern) and the bodies to assume a bivalent appearance at the anterior end of the nucleus. The middle-piece becomes drawn out into a granular thread continuous with the axial filament, while the acrosome appears later and fuses with the other two bodies.

It would appear that the chromatin-staining bodies, to which the axial filament is attached, must be the centrosomes, and that their change in position, due to the rotation of the nucleus, is exactly parallel with what occurs in *Lepisma domestica*.

While no opinion as to the origin of the acrosome is advanced, the fact that it arises in relation to the granules from which the axial filament originally developed would point to the probability of a centrosomal origin. The granular thread which he considers to be the drawn-out middle-piece is, in *Lepisma domestica*, simply the proximal end of the axial filament carried to the apex of the cell along with the centrosome, and it would seem as though such an interpretation could be made of Goldsmith's results.

#### *The middle-piece*

Wilson ('06, p. 337), in speaking of the essential structures in a spermatozoon, lists the middle-piece as a body which "either contains a formed centrosome or a pair of centrosomes, or is itself a metamorphosed centrosome."

It is altogether possible that the middle-piece in *Lepisma domestica* arises from one or both of the granules which we found to have their origin from the centrosome and later traced them to where they were closely applied to the nuclear wall. In fact, we would naturally expect something of the kind, but unfortunately the later history of the granules could not be followed. The question is further confused by the presence of a body which condenses from the nebenkern ring and which, from its size, staining power, and position, would point to its becoming the middle-piece. Furthermore this body can be found practically in all the stages up to the apposition of the middle-piece to the nucleus, but here again we are stopped, for we have not seen the actual formation of this body into the middle-piece. It is possible that a further study will solve this difficulty.

#### *Comparison with Orthoptera*

At the first glance there seems to be a similarity between the spermatogenesis in *Thysanura* as described above and that in *Orthoptera* as previously described and particularly given by Payne ('16) for *Grylotalpa borealis*, but there are also differences which cause one to question whether the resemblance is not more superficial than real.

In the first place, the general shape and arrangement of the chromosomes in the spermatogonia of *Gryllotalpa borealis* are very much like similar stages in *Lepisma domestica*. Payne also has described changes in the mitochondrial mass of the spermatid (his figures E, F, and G, pl. 2), which have almost exact counterparts in *Lepisma domestica*. Then again his figure J on plate 3 shows an axial filament in which the cytoplasm is so arranged in waves as to look like the undulating membrane found in the *Thysanura*.

A comparison of the group of chromosomes associated probably with sex in the two forms shows, however, several important differences. In *Gryllotalpa borealis* Payne finds a single chromosome which does not divide in the first maturation division and therefore could be directly compared with the idiochromosomes of the *Thysanura* were it not for the fact that the single chromosome is associated with an unequal bivalent chromosome and in division always goes to the same pole as the large 'end' of the unequal chromosome. Therefore, the two resulting secondary spermatocytes differ not only in that one has an extra chromosome, but also in that the same cell possesses the large 'end' of the unequal chromosome, while the smaller part passes to the other cell. Payne favors the view that these chromosomes represent a triad group rather than an unequal pair of idiochromosomes and an accessory chromosome.

Payne has not been able to trace the centrosome of the second maturation division through to the spermatid, and in fact has not been able to demonstrate the presence of the centrosome in the spermatid at all, although he presumes that the body from which the axial filament arises and which later becomes the middle-piece may be a centrosome. Further, he describes the acrosome as arising from an elongated body which suddenly appears *de novo* in the cytoplasm, whereas in *Lepisma domestica* the acrosome is formed from a rod-like centrosome.

## SUMMARY

1. The male *Lepisma domestica* has three pairs of testes on each side, each testis connected by a duct with the respective vas deferens.
2. The blind end of the testis contains the youngest stages.
3. Primary spermatogonia are formed very early in life.
4. Thirty-four chromosomes are present in the spermatogonia. A chromatic nucleolus is present in the resting stage of the spermatogonia.
5. The growth stages follow the description given by Wilson ('12).
6. A planosome is seen in the resting stage of the spermatogonia appearing in the growth stages as a much larger body.
7. One or two plasmosomes appear shortly after formation of the spireme threads, and disappear later.
8. There are eighteen chromosomes in the first maturation division. The two idiochromosomes pass undivided to one pole.
9. The autochromosomes divide longitudinally in the second division, while the idiochromosomes do not. Instead, they separate, each spermatid receiving one idiochromosome.
10. The form of the centrosome is changeable, but its almost constant presence either in the shape of a granule or of a rod indicates that it may be a permanent cell structure.
11. A chromatic nucleolus is present in half of the spermatids.
12. The nebenkern is formed from granular mitochondria, the remains of the last and of two previous spindles.
13. The axial filament grows from the end of the rod-shaped centrosome which forms the acrosome.
14. Another body, presumably derived from the nebenkern, forms the middle-piece.
15. The nebenkern, after separating out the spindle remains and several accumulations of mitochondrial material, form a vacuolated body which furnishes a sheath for the axial filament.
16. The axial filament persists and forms the undulating membrane of the mature spermatozoa.

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## EXPLANATION OF PLATES

All the figures, with the exception of the first three, were made with a Zeiss 2-mm. apochromat. objective and Zeiss compensating ocular no. 12. In order to get as high a magnification as possible, drawings were made at table level, giving an enlargement of about 1850 diameters.

### ABBREVIATIONS

<i>a</i> , acrosome	<i>p</i> , penis
<i>af</i> , axial filament	<i>pl</i> , plasmosome
<i>c</i> , centrosome	<i>ps</i> , planosome
<i>i</i> , idiochromosomes	<i>s</i> , spindle remains
<i>m</i> , mitochondria	<i>sv</i> , seminal vesicle
<i>mp</i> , middle-piece	<i>t</i> , tubules
<i>n</i> , nebenkern	<i>vd</i> , vas deferens
<i>o</i> , ovipositor	<i>x</i> , middle-piece anlage

## PLATE 1

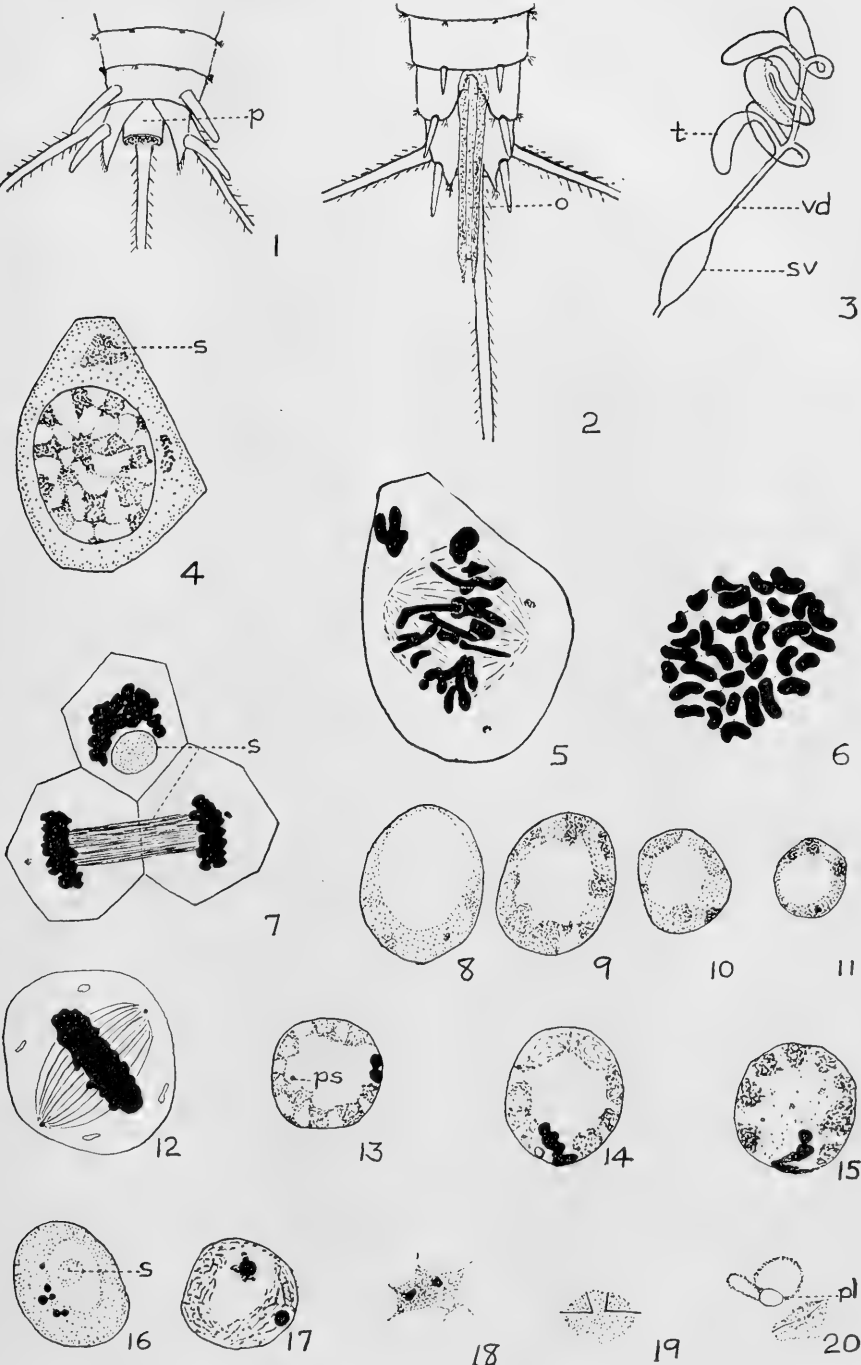
### EXPLANATION OF FIGURES

- 1 Posterior segments, ventral surface, of male *Lepisma domestica*.  $\times 20$ .
- 2 Posterior segments, ventral surface, of female *Lepisma domestica*.  $\times 20$ .
- 3 Testes of one side showing connection with vas deferens and seminal vesicle.  $\times 20$ .
- 4 Spermatogonium, surface view, showing resting condition of nucleus and the attraction sphere.
- 5 Prophase of spermatogonium.
- 6 Metaphase plate early spermatogonium. Thirty-four chromosomes joined by linin threads.
- 7 Telophase spermatogonia showing persistent spindle remains.
- 8, 9, 10, and 11 Nuclei of spermatogonia decreasing in size with each division.
- 12 Spermatogonial metaphase from the side. Centrosome as single granule at poles.
- 13, 14, and 15 Resting stages spermatogonia to show idiochromosomes.
- 16 Spermatogonium showing the breaking up of the idiochromosomes.
- 17 Beginning of growth period. Idiochromosomes reformed.
- 18 Spindle remains from spermatogonium with two centrosome granules.
- 19 Same as the preceding from early growth period with V-shaped centrosomes.
- 20 Centrosome rods with granules at their ends, before division. Plasmosome and idiochromosome loops.

SPERMATOGENESIS OF LEPISMA DOMESTICA

HARRY H. CHARLTON

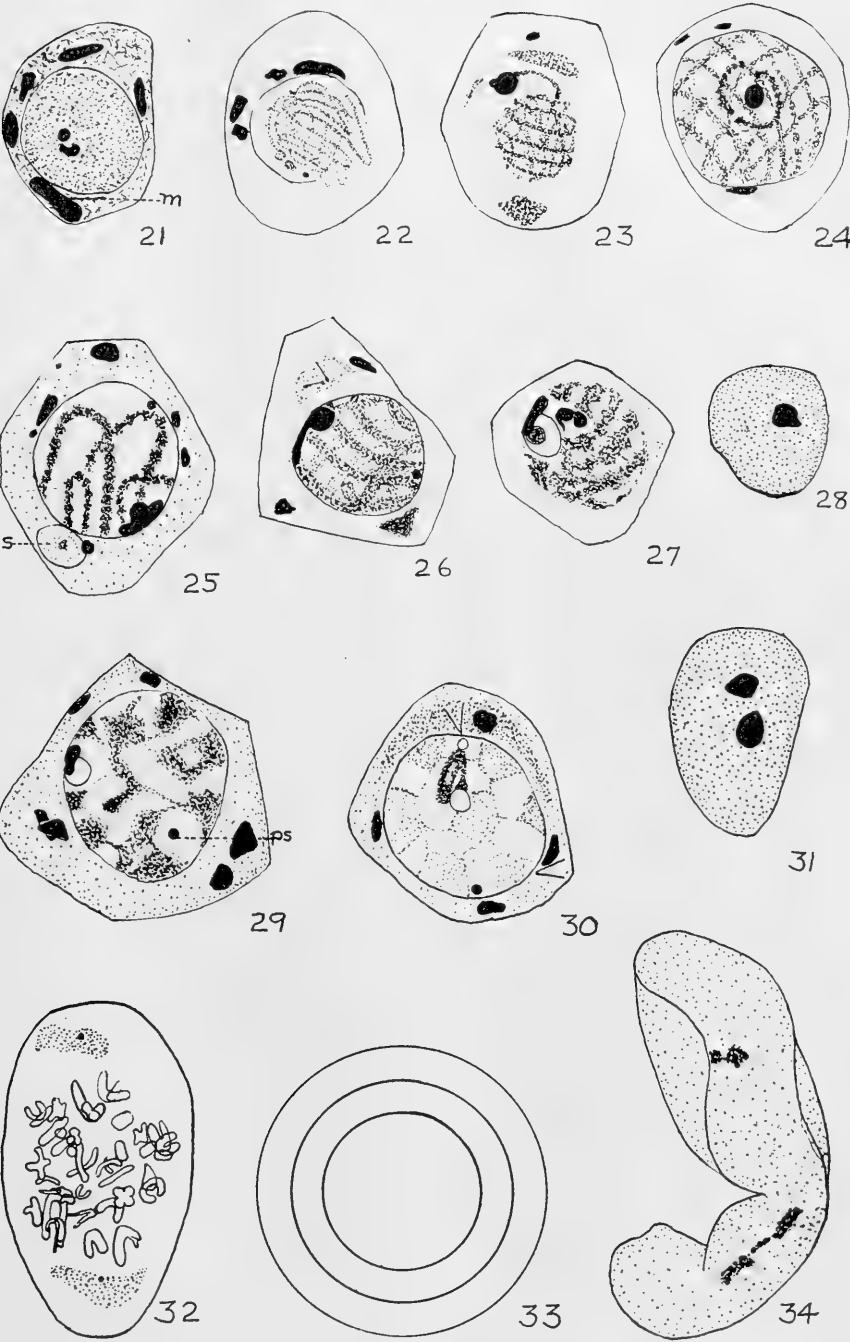
PLATE 1



## PLATE 2

### EXPLANATION OF FIGURES

- 21 First spermatocyte. Nucleus homogeneous except for idiochromosomes.
- 22 First spermatocyte showing fine, loosely arranged leptotene thread. Idiochromosomes very pale in color.
- 23 Early contraction stage of first spermatocyte. Plasmosomes with idiochromosome thread to one side.
- 24 First spermatocyte showing a spreading out of the threads following contraction. Plasmosome and idiochromosome threads shown in surface view.
- 25 Bouquet stage of first spermatocyte. Only a few loops shown in drawing.
- 26 Pachytene stage of first spermatocyte.
- 27 Same as the preceding, only somewhat later.
- 28, 31, and 34 Sertoli cells associated with different stages in the development of the spermatozoa.
- 29 Confused or net-like stage of first spermatocyte. Planosome prominent.
- 30 Early prophase of first spermatocyte. Centrosomes have migrated nearly to poles. Two plasmosomes present.
- 32 Dividing cell from spermatogonial region. Possibly a Sertoli cell. Chromosomes paired.
- 33 Diagram to show the relative sizes of cells of first and second maturation divisions and of the spermatid.



## PLATE 3

### EXPLANATION OF FIGURES

35 The plasmosomes and idiochromosomes during the growth period of the first spermatocyte showing formation of spireme threads and their contraction into the prochromosomes.

36 and 37 Prophase of first spermatocyte showing connected idiochromosomes.

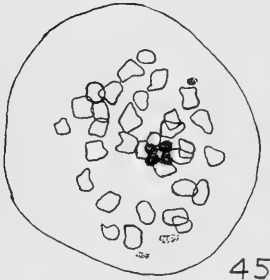
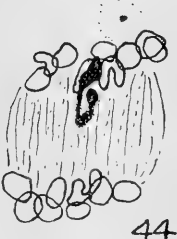
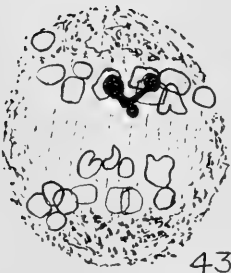
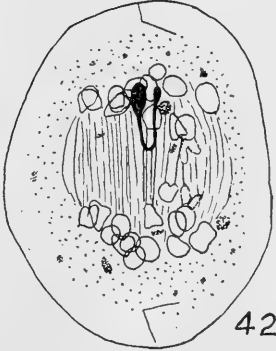
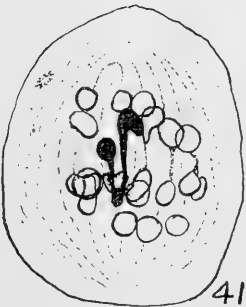
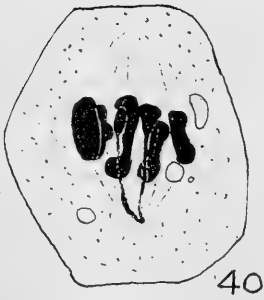
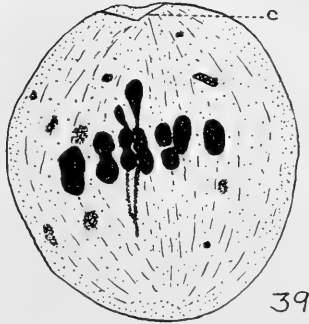
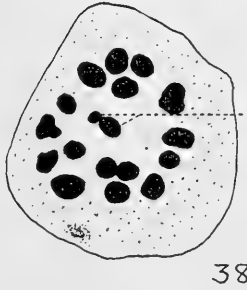
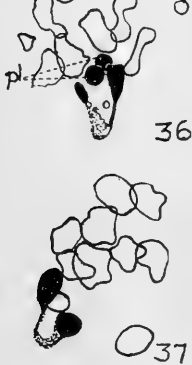
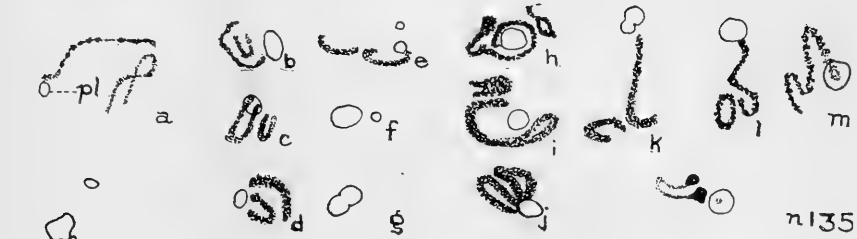
38 Metaphase plate of first spermatocyte with idiochromosomes in center.

39 Side view metaphase of first spermatocyte to show dumb-bell-shaped chromosomes and their transverse division. Idiochromosomes joined together.

40 Same as the preceding.

41, 42, 43, and 44 Side views of dividing first spermatocyte, showing idiochromosomes during anaphase and telophase.

45 Oblique view of anaphase of dividing first spermatocyte. Thirty-three chromosomes plus the idiochromosome complex which here shows each idiochromosome as bivalent.

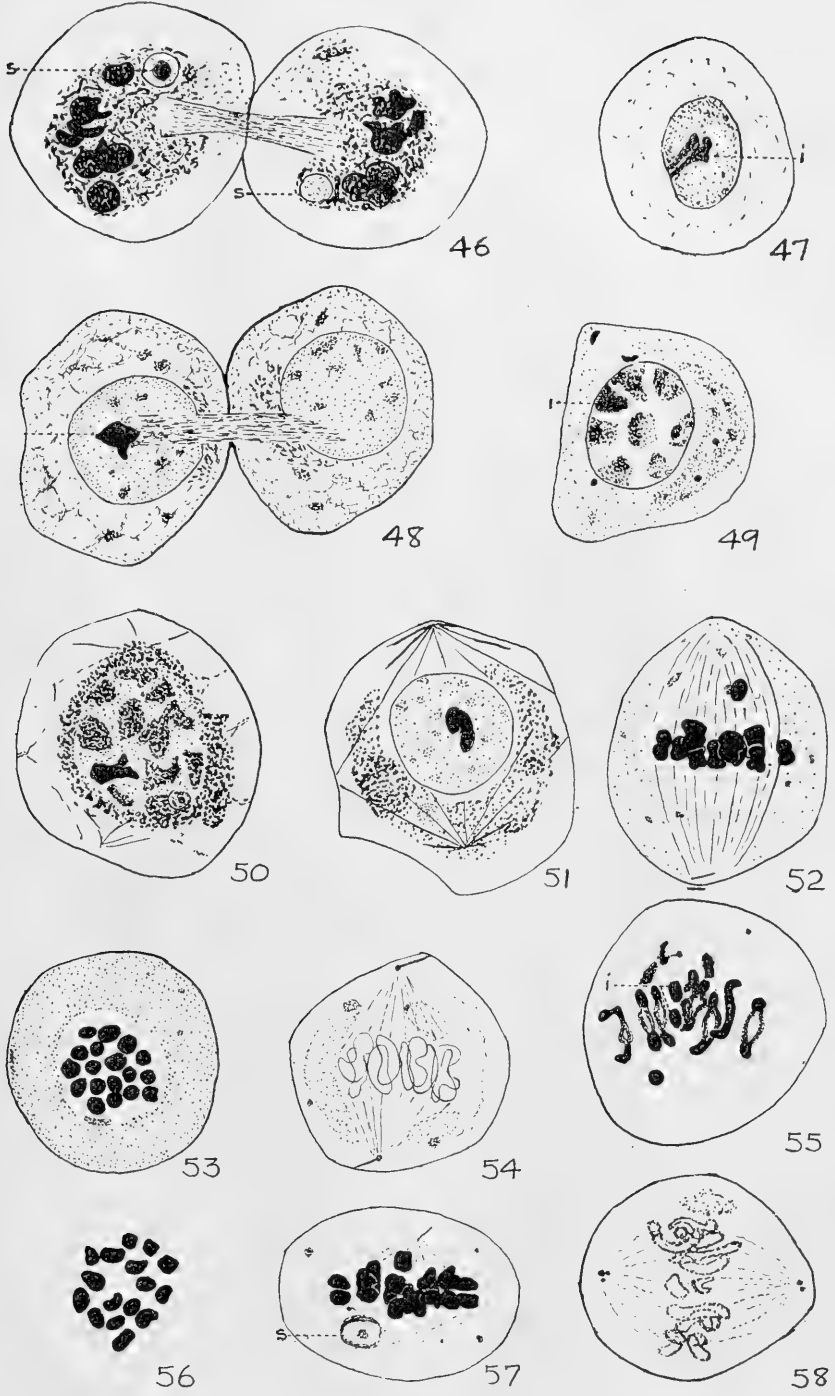


## PLATE 4

### EXPLANATION OF FIGURES

- 46 Early second spermatocyte, showing the breaking up of the telophase chromosomes. Position of spindle remains typical.
- 47 Resting stage of second spermatocyte.
- 48 Same as the preceding, the chromatic nucleolus found in only half the cells of the second spermatocyte.
- 49 Early prophase of second spermatocyte.
- 50 Same as the preceding, chromatic nucleolus double. Ring of mitochondrial granules around the nucleus.
- 51 Resting stage of second spermatocyte, showing formation of spindle fibers before the prophase stage is reached.
- 52 Side view metaphase of second spermatocyte.
- 53 Eighteen chromosome metaphase plate of the second spermatocyte.
- 54 Side view metaphase plate of second spermatocyte. Rod centrosomes with granules at inner ends.
- 55 Early anaphase of second spermatocyte with the autochromosomes dividing longitudinally, while the idiochromosomes have separated transversely.
- 56 Metaphase plate of second spermatocyte. The sixteen chromosome type.
- 57 Later anaphase of second spermatocyte.
- 58 Same as the preceding with granules at poles instead of rods.

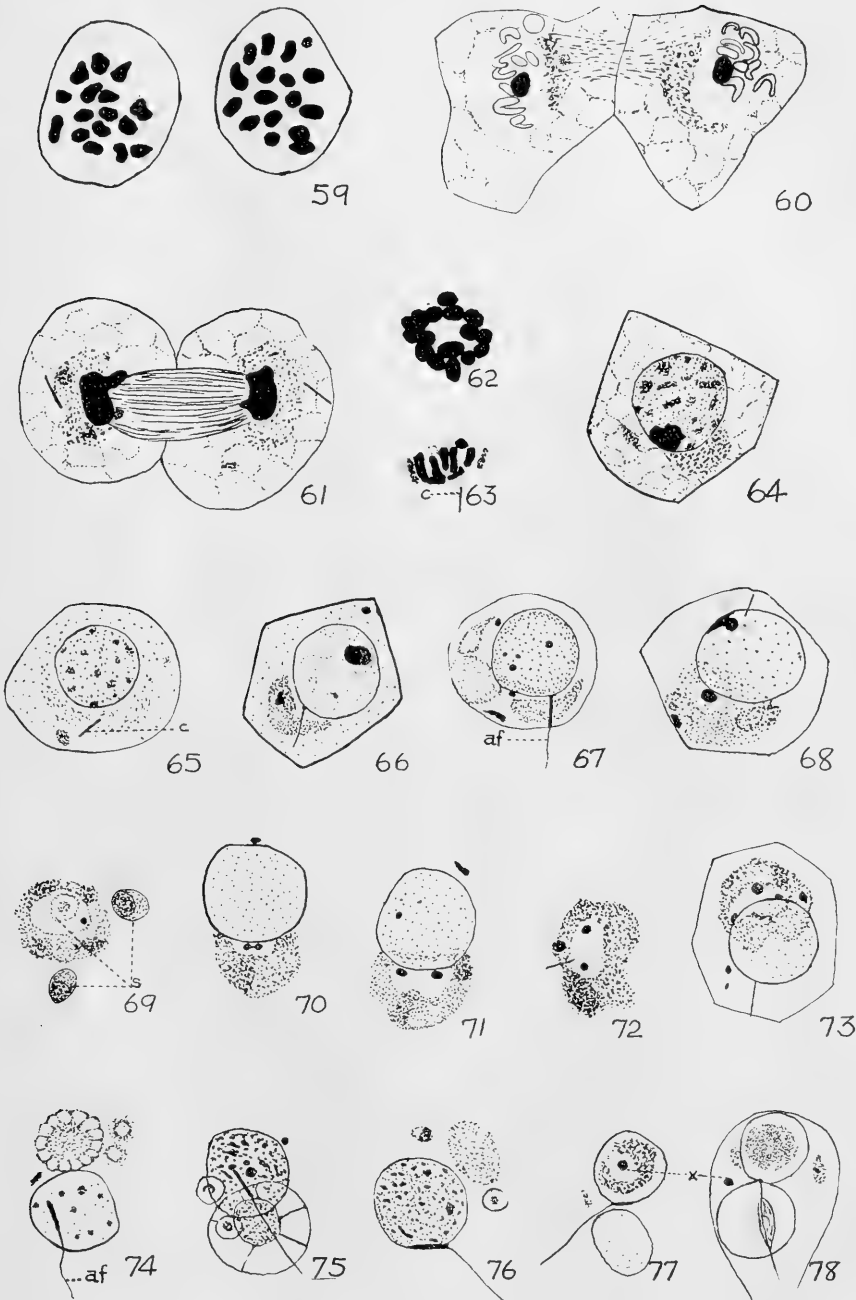




## PLATE 5

### EXPLANATION OF FIGURES

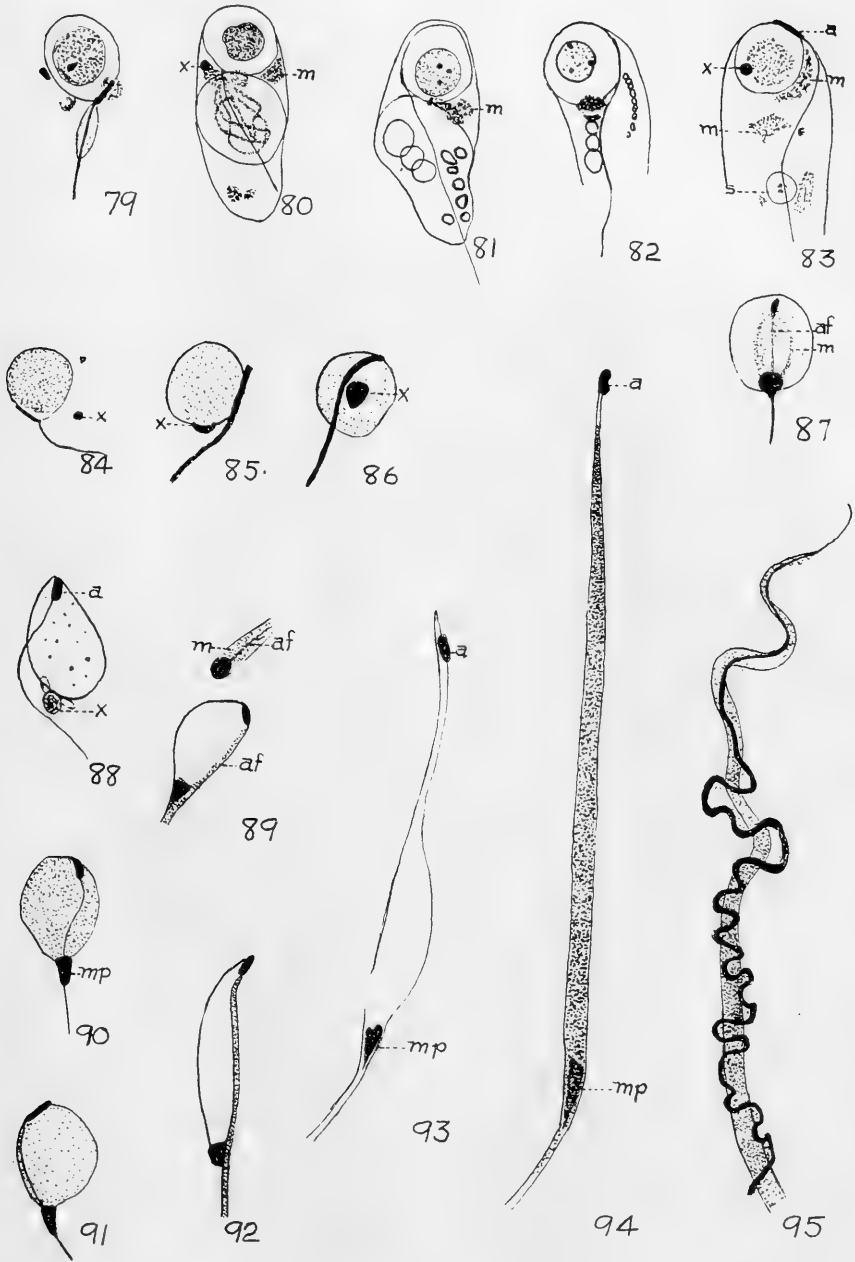
- 59 Two anaphase drawings of second spermatocyte from same cell. Sixteen chromosomes in each.
- 60 Spermatids from division of eighteen chromosome second spermatocyte. The divided idiochromosomes differ in shape and are darker stained than the autochromosomes.
- 61 Young spermatids before the reorganization of nucleus.
- 62 Anaphase of second spermatocyte, showing ring-like arrangement of chromosomes.
- 63 Early formation of nucleus of spermatid.
- 64 Spermatid with resting nucleus and nucleolus.
- 65 Early spermatid with rod centrosome.
- 66 Same as the preceding, showing centrosome with granule at base lying against nuclear membrane.
- 67 Same as the preceding. The granule broken off.
- 68 Same as the preceding. The granule much enlarged.
- 69 Elements of nebenkern from early spermatid.
- 70 Spermatid showing division of granule.
- 71, 72, and 73 The nebenkern ring from spermatid, showing separation of granules and formation of new body from the nebenkern.
- 74 Central part of nebenkern of spermatid, showing rosette form and spindle remains.
- 75 Spermatid showing vacuolization of nebenkern border further advanced. Nuclear granules larger and stain darker.
- 76 Spermatid showing centrosome applied to nuclear membrane.
- 77 and 78 Spermatid showing clear space forming around chromatin of nucleus. Middle-piece anlage present.



## PLATE 6

### EXPLANATION OF FIGURES

- 79 Same stage as figure 78. Centrosome rod shown clearly.
- 80 Spermatid with chromatin of nucleus more condensed. Thread-like structure in middle of nebenkern vacuole. Mitochondria between nebenkern and nucleus as well as distal to nebenkern.
- 81 Same as the preceding, but threads of nebenkern have broken and now form vesicles.
- 82 Same as the preceding showing linear arrangement of small vesicles.
- 83 Spermatid of a slightly later state than the preceding.
- 84 Spermatid with nebenkern contents thrown off.
- 85 and 86 Spermatids. Side and surface view adjacent cells to show relation of axial filament to middle-piece anlage. Filament thicker on account of mitochondrial granules in relation to it.
- 87 Spermatid showing mitochondrial granular mass in relation to middle-piece and axial filament.
- 88 Spermatid showing secondary relationship to middle-piece anlage.
- 89 Spermatid. A later stage to show mitochondrial ribbon about the proximal part of the axial filament.
- 90 Spermatid nucleus elongating and staining darker. Middle-piece getting longer.
- 91 Same as the preceding.
- 92 Elongation of nucleus of spermatid. Mitochondria about filament.
- 93 Spermatid. A still later stage, no mitochondria shown.
- 94 Spermatid nucleus very much drawn out. Acrosome at apex and darker part indicates position of middle-piece.
- 95 Part of anterior end of mature spermatozoon. The undulating membrane with its thicker border is well shown.



Resumen por el autor, James Ernest Kindred,  
Western Reserve University.

### El condrocráneo de *Syngnathus fuscus*.

*Syngnathus fuscus*, un representante de los Lofobranquios, se asemeja a *Gasterosteus*, un hemibranquio, en los siguientes caracteres condrocraneales: La presencia de una región etmoidal alargada; la articulación acartética de los cartílagos palatinos; el techo craneal incompleto; la posición horizontal de las trabéculas del cráneo y cartílagos paracordales; la presencia de dos tabiques semicirculares en cada cápsula ótica; un proceso protótico que separa los nervios trigémino y facial; una ventana basieraneal posterior alargada; un orificio común para los nervios glosofaríngeo y vago situado entre la cápsula ótica y el arco occipital; un cartílago ptérigo-cuadrado pequeño; un voluminoso notocordio intercraneal y un proceso post-orbitario en el borde anterior de cada cápsula ótica. Las especializaciones observadas en el condrocráneo de los estados de 8 by 12 mm. de *Syngnathus* son: La elevación dorsal del extremo anterior de la placa etmoidea en el estado de 8 mm.; la presencia de una ventana miódica ventral definida; una cinta tectal mediana en el techo craneal; una pequeña ventana basicapsular en la pared ventral de cada cápsula ótica; una conexión fibrosa entre los cartílagos palatino y pterigoideo; la notable longitud de la región etmoidal; el cambio de posición de los elementos hioideos al comenzar a funcionar las branquias; la presencia de un proceso metapterigoideo reducido; y la ausencia de cartílagos dorsal y ventral en el notocordio. Los siguientes caracteres primitivos importantes son dignos de mención: Presencia de un cartílago rostral independiente, el cual se ha considerado como el homólogo de la sincondrosis entre los extremos distales de los palatinos de *Heptanchus*; el desarrollo de los cartílagos ectetmoideos independientes del etmoides; la comunicación abierta entre la cavidad del laberinto y la del cráneo, y la ausencia de nervios post-vagos en la región occipital.

THE CHONDROCRANIUM OF SYNGNATHUS FUSCUS

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FOURTEEN FIGURES

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INTRODUCTION

The study of the chondrocranium of *Syngnathus fuscus* was undertaken at the suggestion of Prof. J. S. Kingsley upon the completion by the author of a monograph on the skull of *Amiurus*. A detailed account of the facts of development of the chondrocranium of *Syngnathus* had not as yet been given. McMurrich's ('83) paper, which contains a general description of the development of the chondrocranium of *Syngnathus peckianus*, has served as a basis for our knowledge of the cranium of the *Lophobranchii*, the group of teleosts of which *Syngnathus* is a representative. In order to add to the knowledge of the cranial characters of this group, the following detailed topographical and histological description of the early stages in the development of the chondrocranium of *Syngnathus fuscus* is presented.

It is the purpose of the author to describe in a subsequent paper the later stages in the development of the chondrocranium which lead to the formation of the adult skull.

## MATERIAL AND METHODS

The description of the chondrocranium of *Syngnathus fuscus* is based upon the study of serial sections through the head regions of embryos from 6 mm. to 15 mm. in length. For purposes of orientation and comparison, reconstructions were made in wax of the chondrocrania of the 8-mm. and 12-mm. embryos, which represent typical stages in its development.

Male pipe-fish carrying young in the brood pouch were kept in laboratory aquaria, and a few embryos from each fish were taken out and fixed daily. It is impossible to state the exact age in hours of the embryos, because in all cases where they were collected, development was advanced at least as far as the closure of the neural tube. The 12- to 15-mm. stages were larvae capable of caring for themselves. These were the oldest animals that I was able to raise in the laboratory, because their resistance is very low to changes in the environment at this age, the critical period when the yolk sac has just been absorbed.

The material for study was collected at Woods Hole during the summer of 1919 by the Supply Department of the Marine Biological Laboratory.

## THE CHONDROCRANIUM OF THE 8-MM. STAGE

In embryos of *Syngnathus fuscus* younger than the 8-mm. stage of development, the chondrocranium has not been definitively laid down. The cranial flexure is marked and the head region has not straightened out. In a 6-mm. embryo the visceral arches are formed of dense mesenchymatous masses; procartilage tracts are present ventrolateral to the otic vesicles, but were not observed ventral to the brain or lateral to the notochord. Since chondroblasts are not present except in these regions, it is probable that the chondroblasts which later form the basis cranii are proliferated from the visceral-arch masses of mesenchyme.



*A. The neurocranium*

In the neurocranium of the 8-mm. embryo, the parts typical of a teleostean neurocranium are present, but are modified somewhat, foreshadowing even thus early the elongate character of the adult cranium. The elongation seems to be due to a precocious growth of the cartilage forming the trabecula communis, since the distance from the anterior end of the ethmoid plate to the anterior margin of the fenestra myodome ventralis is about two-thirds of the total length of the cranium (fig. 2), whereas in a 25-mm. *Salmo* (Gaupp, '06) it is equal to one-half, and in a 6.6-mm. *Gasterosteus* (Swinerton, '02), to one-third of the total length of the cranium.

The anterior half of the ethmoid plate turns abruptly dorsal, making a right angle with its proximal portion (fig. 1). The dorsally turned part of the ethmoid is flatter in cross-section than is its horizontal portion and is expanded laterally at its dorsal end to form the rostral process (fig. 3). The lateral surface of each side of the rostral process is grooved to receive the proximal end of the palatine cartilage of that side.

In the chondrocrania of other teleosts which have been described there is no reference to a dorsal turning of the anterior ethmoidal region. Swinerton has noted a widening of the ethmoid plate and its articulation with the palatoquadrate in the 5.7-mm. *Gasterosteus*. The articular processes which are the homologues of those in *Syngnathus* he named the pre-ethmoid cornua. He called this type of articulation, *acrartete*; "a palato-ethmoidal relationship in which the attachment of the palatine cartilage is confined solely to the pre-ethmoid cornua."

A small median rostral cartilage lies on the dorsal surface of the rostral process. It is attached to it by densely cellular connective tissue, but otherwise is independent of the surrounding structures (figs. 1, 2, 3). The phylogenetic significance of this cartilage will be considered in the description of the visceral arches.

Just posterior to the rostral region of the ethmoid plate, the plate proper is broad and ovoid in cross-section. It is continuous

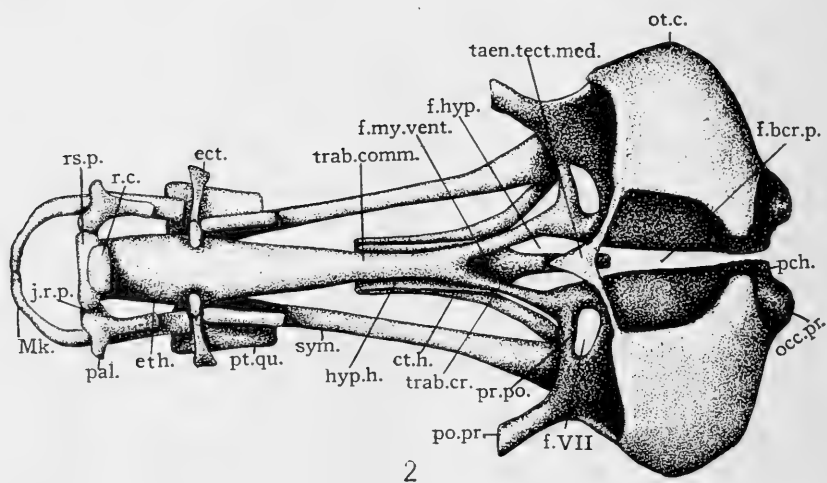
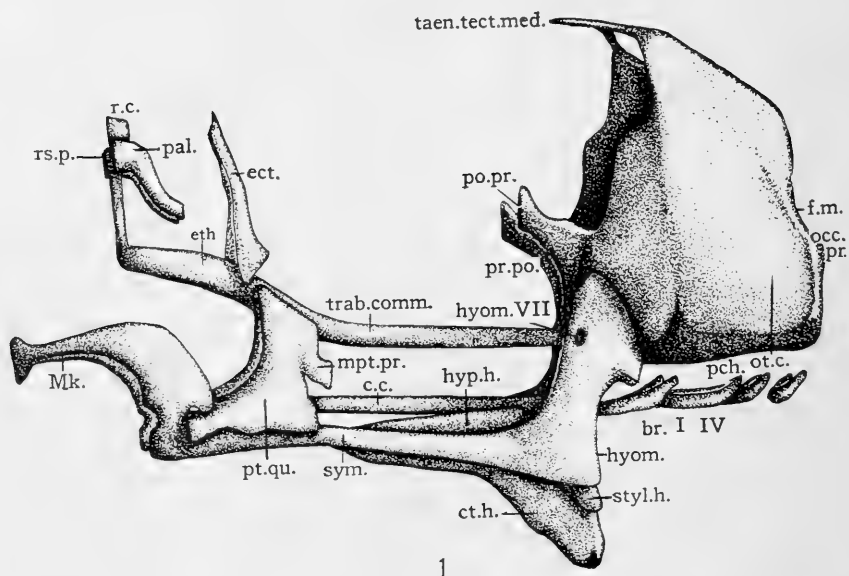


Fig. 1 Lateral view of the chondrocranium and visceral arches of an embryo of *Syngnathus fuscus* 8 mm. long. Drawing made from a wax model 230 times the actual size of the chondrocranium. Ratio of drawing to model, 2:3.

Fig. 2 Dorsal view of the same model as shown in figure 1.

posteriorly with the trabecula communis which forms the axis of the orbital region. The interorbital septum of loose strands of fibrous connective tissue is directly continuous with the perichondrium of the ethmoid plate (fig. 4). The olfactory pits lie lateral to the interorbital septum and are embedded in embryonal connective tissue.

Just posterior to the olfactory pits and ventral to the anterior end of the brain there is a pair of cartilaginous rods. These extend ventrolaterally from the membranous tissue enclosing the brain into the embryonal connective tissue lying in the anterior portion of the orbit (fig. 1). McMurich, in describing the chondrocranium of a 6- to 7-mm. *Syngnathus peckianus*, interpreted these as the nasal cartilages. In other teleosts the ectethmoid cartilages occur in this region as lateral outgrowths from the ethmoid plate. In later stages of *Syngnathus* the ectethmoid processes are completely fused with the ethmoid plate, the whole forming a mesial internasal septum. Hence from their position and their later history these independent cartilages are to be regarded as ectethmoid cartilages, but at the same time this condition indicates that the ectethmoid cartilages were derived phylogenetically from the posterior wall of the cartilaginous olfactory capsules and that only a remnant of these is left in *Syngnathus*. If this point of view is accepted,

## ABBREVIATIONS

<i>br.I-IV</i> , branchial arches I to IV	<i>Mk.</i> , Meckel's cartilage
<i>c.c.</i> , copula communis	<i>mpt.pr.</i> , metapterygoid process
<i>ct.h.</i> , cerato hyal	<i>occ.pr.</i> , occipital process
<i>ect.</i> , ectethmoid cartilage	<i>ot.c.</i> , otic capsule
<i>eth.</i> , ethmoid plate	<i>pal.</i> , palatine cartilage
<i>f.bcr.p.</i> , fenestra basicranii posterius	<i>pch.</i> , parachordal
<i>f.hyp.</i> , fenestra hypophyseos	<i>po.pr.</i> , postorbital process
<i>f.my.vent.</i> , fenestra myodomeus ventralis	<i>pr.po.</i> , prootic process
<i>f.m.</i> , foramen magnum	<i>pt.qu.</i> , pterygoquadrate cartilage
<i>f.VII</i> , foramen for ramus hyomandibularis facialis	<i>r.c.</i> , rostral cartilage
<i>Hyom. VII</i> , foramen in hyomandibula for r.hyom.fac.	<i>rs.p.</i> , rostral process of ethmoid
<i>hyp.h.</i> , hypohyal	<i>styl.h.</i> , stylohyal cartilage
<i>j.r.p.</i> , junction rostopalatinus	<i>sym.</i> , symplectic cartilage
	<i>taen.tect.med.</i> , taenia tectum medialis
	<i>trab.comm.</i> , trabecula communis
	<i>trab.cr.</i> , trabecula cranii

then it may be stated that the relation of the ectethmoid cartilages to the ethmoid plate is more primitive in *Syngnathus* than in *Gasterosteus*, where they are never independent.

The tegmen cranii of the ethmoid region described and figured for *Syngnathus peckianus* by McMurrich is not present, the brain is enclosed in a fibrous connective-tissue sheath alone (fig. 4).

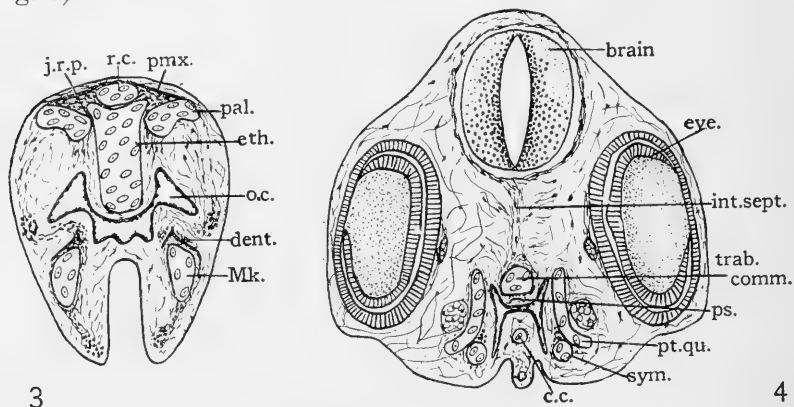


Fig. 3 Cross-section through the anterior end of the ethmoid region of the 8-mm. *Syngnathus*. Semidiagrammatic. Camera lucida.  $\times 100$ .

Fig. 4 Cross-section through the anterior end of the orbit of an 8-mm. *Syngnathus*. Semidiagrammatic. Camera lucida.  $\times 100$ .

#### ABBREVIATIONS

*c.c.*, copula communis  
*dent.*, dentary ossification  
*eth.*, ethmoid plate  
*int.sept.*, interorbital septum  
*j.r.p.*, junction rostopalatinus  
*Mk.*, Meckel's cartilage  
*o.c.*, oral cavity

*pmx.*, premaxillary ossification  
*pal.*, palatine cartilage  
*ps.*, parasphenoid ossification  
*pt.qu.*, pterygoquadrate cartilage  
*r.c.*, rostral cartilage  
*sym.*, symplectic cartilage  
*trab.*, trabecula communis

Posterior to the ectethmoid cartilages, the interorbital septum becomes broader and appears as a meshwork of connective-tissue fibers within which are scattered numerous stellate cells. If we assume with Swinnerton that the ethmoid region ends with the posterior end of the ectethmoid cartilages, then it may be stated that dorsally this meshwork is continuous with the membranous sheath surrounding the brain, and ventrally with the perichondrium of the trabecula communis.

As in other non-siluroid teleosts, the trabecula communis is considerably narrowed between the greatly enlarged eyes (figs. 2, 4). While in this region of a 5.7-mm. *Gasterosteus* the brain lies immediately dorsal to the trabecula communis, in *Syngnathus* it is widely separated—a condition which may be ascribed to the more rapid growth of the trabecula communis.

The optic nerves cross through the interorbital septum in the region of the optic chiasma a short distance dorsal to the trabecula communis. The septum is distinct ventral to their crossing. Posterior to this region the interorbital septum is broad and indistinct, and it becomes less apparent as the ventral surface of the brain approaches the trabecula communis in the region just anterior to the fenestra myodorus ventralis.

A cross-section of the trabecula communis in the interorbital region has a distinctly double character, which indicates its formation by the fusion of the anterior ends of the trabeculae cranii. The anterior extent of the fenestra myodorus ventralis is not as great in *Syngnathus* as in a 5.7-mm. *Gasterosteus*, hence the trabecula communis is relatively shorter in the latter.

A mass of diffuse mesenchyme cells, the primordium of the vomer bone, is present along the ventral surface of the ethmoid plate. More posteriorly a convex osseous lamella abuts against the ventral surface of the trabecula communis. This is the first ossification in the cranium and is the beginning of the parasphenoid (fig. 4). It forms the median floor of the fenestra myodorus ventralis and of the fenestra hypophyseos (figs. 5, 6). No mention of this ossification is made in the description of the early stages of the chondrocranium of *Gasterosteus*.

Posterior to the orbital region, the trabeculae cranii diverge to form the margins of the fenestra myodorus ventralis (fig. 5). This fenestra is so named because of its homology to that space in other teleosts, which lies between the trabeculae cranii and anterior to the hypophysis, and lodges the proximal ends of the recti eye muscles (Allis, '19). These eye muscles have their origin on the dorsal surface of the parasphenoid lamella and are not as yet separate elements. Posterior to the origin of the recti muscles, the fenestral space is filled with a dense mass of

connective-tissue cells which is separated from the ventral surface of the brain by a layer of fibrous connective tissue. Throughout this region the trabeculae cranii are circular in cross-section.

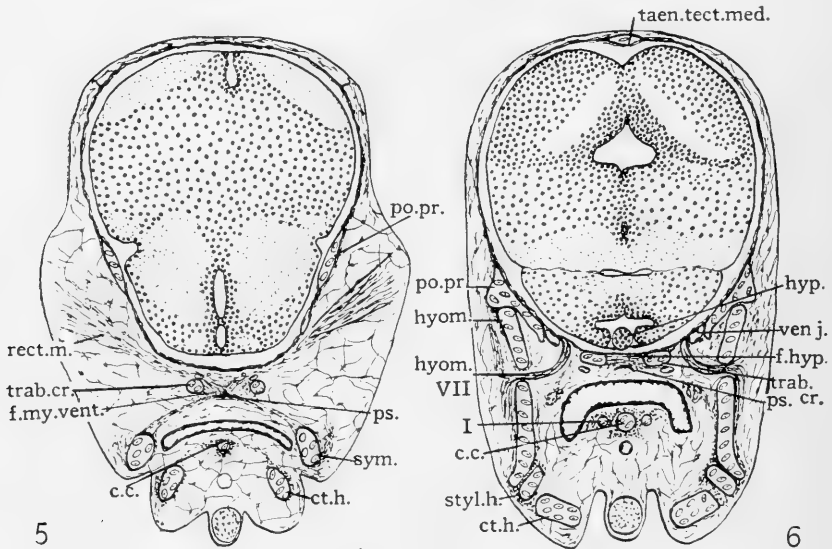


Fig. 5 Cross-section through the posterior part of the orbit, 8-mm. *Syngnathus*. Semi-diagrammatic.  $\times 100$ .

Fig. 6. Cross-section through the anterior end of the otic capsule, 8-mm. *Syngnathus*. Semi-diagrammatic.  $\times 100$ .

#### ABBREVIATIONS

<i>br.I</i> , branchial arch I	<i>po.pr.</i> , postorbital process
<i>c.c.</i> , copula communis	<i>po.pr.</i> , postorbital process
<i>ct.h.</i> , ceratohyal cartilage	<i>ps.</i> , parasphenoid ossification
<i>f.hyp.</i> , fenestra hypophyseos	<i>rect.m.</i> , recti eye muscles
<i>f.my.vent.</i> , fenestra myodome ventralis	<i>styl.h.</i> , stylohyal cartilage
<i>hyom.</i> , hyomandibula	<i>sym.</i> , symplectic cartilage
<i>hyom.VII</i> , ramus hyomandibularis facialis	<i>taen.tect.med.</i> , taenia tectum medialis
<i>hyp.</i> , hypophysis cerebri	<i>trab.cr.</i> , trabecula cranii
	<i>ven.j.</i> , vena jugularis

As the divergence of the trabeculae becomes greater, the parasphenoid lamella remains as a small spicule in the median part of the fenestra and is widely separated from the trabeculae cranii. The gasserian ganglion lies in a space between each

trabecula cranii and the postorbital process of that side. Swinnerton indicates this relation of the trigeminal ganglion to the trabecula and the postorbital process in *Gasterosteus*. Gaupp calls this space in a 25-mm. *Salmo* the incisivum prooticum.

Farther posterior, the prootic process of each side abuts against the lateral surface of the trabecula cranii (figs. 1, 2). The cartilage forming each of these structures retains its identity at the point of union. The perichondria of the two cartilages form the line of separation between them. In this immediate region the trabeculae are flatter and more ovoid than they are more anteriorly. This is probably the region of junction between the trabeculae and the parachordal cartilages (fig. 6).

A wide space, such as is found between the trabeculae cranii and the ventral surface of the brain in the fenestra myodome ventralis region, is obliterated here by the presence of the hypophysis cerebri, so that the membrane enclosing the brain is continuous with the perichondria of the trabeculae cranii (fig. 6).

A foramen is present in the cranial wall just posterior to the prootic process. Through this foramen the jugular vein and the ramus hyomandibularis facialis pass (fig. 6). The posterior margin of the foramen is formed by the wall of the otic capsule.

Posterior to the facialis foramen the parachordal cartilages are fused with the ventromesial margins of the otic capsules. Mesially, the parachordals are closer together than were the trabeculae farther anterior. The parasphenoid lamella forms the floor of the intervening fenestra, its roof is formed by fibrous connective tissue ventral to the posterior end of the hypophysis.

Just posterior to this region the parasphenoid ends and the space between the parachordals is occupied by the anterior end of the notochord (fig. 7). The notochord is separated dorsally from the cavum cranii by connective-tissue stroma connecting the perichondria of the parachordals. The space between the parachordals in which the notochord lies has been called the inter-parachordal fossa in *Gasterosteus* (Swinnerton), and the fenestra basicranii posterius in *Salmo* (Gaupp); the latter terminology is used in this paper.

The cartilage of this region of the parachordals has the same relation to the notochord in both *Syngnathus* and the 5.7-mm. *Gasterosteus*, but it is not as widely separated from the notochord as it is in a 19-mm. *Amia* (Kindred, '19). This indicates

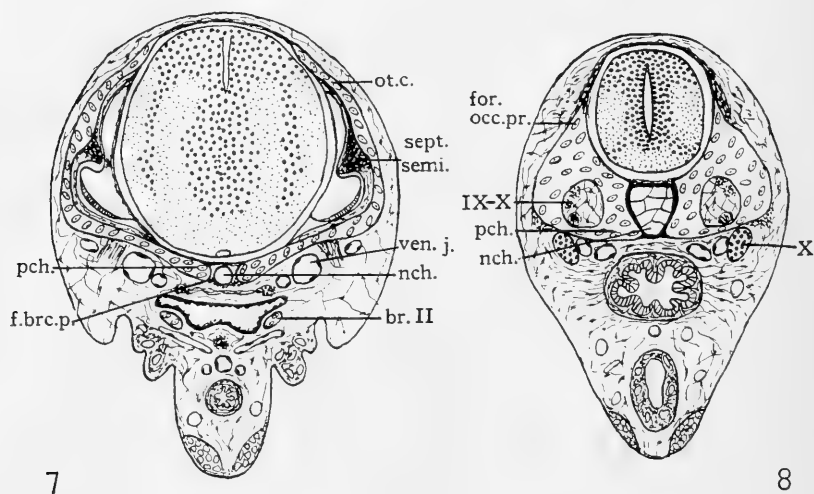


Fig. 7 Cross-section through the middle part of the otic capsules, 8-mm. *Syngnathus*. Semidiagrammatic. Camera lucida.  $\times 100$ .

Fig. 8 Cross-section through the occipital region, 8-mm *Syngnathus*. Semidiagrammatic. Camera lucida.  $\times 100$ .

#### ABBREVIATIONS

*br.II*, branchial arch II

*f.bcr.p.*, fenestra basicranii posterius

*for.IX-X*, foramen for IX and X cranial nerves

*nch*, notochord

*ot.c.*, otic capsule

*pch.*, parachordal cartilage

*sept.semi.*, septum semicircularis lateralis

*ven.j.*, vena jugularis

*X*, ganglion of vagus nerve

a precocious growth of the cartilage in this region of *Syngnathus*.

Laterally, the cartilage of each parachordal is confluent with the ventromesial margin of the otic capsule (fig. 7). The cartilage cells of the parachordals are arranged concentrically, while those of the otic capsule are in a vertical row.



Farther posterior the notochord increases greatly in diameter and invades the cavum cranii. The parachordals abut against the ventrolateral surfaces of the notochord, so that it really lies in a groove on the dorsomesial surfaces of the parachordals. The parachordals are separated from each other ventral to the notochord and there is no trace of a hypochordal bridge.

In the region between the posterior parts of the otic capsules, the enlarged notochord lies between the maculi utriculi and the auditory ganglia adjacent to them ventrolaterally. The relation of the parachordals to the notochord remains as in the more anterior region. At the posterior end of the otic capsules the cartilage forming their walls fuses with the occipital processes which lie on the dorsolateral surfaces of the notochord dorsal to the parachordals (fig. 8). The parachordals retain their identity for a short distance posterior to this fusion; gradually, however, the cells of the parachordals become confluent with those of the occipital cartilages (fig. 8). A slender bridge of fibrous connective tissue connects the occipital processes with each other dorsal to the notochord, but their ventral margins are separated by the notochordal sheath (fig. 8). A canal extends ventroposteriorly from the floor of the otic capsule and opens to the exterior posteroventral to its wall. The mesial wall of this canal is formed by the occipital cartilage (fig. 8). The canal contains a part of the sacculus and the fibers of the glossopharyngeal and vagus nerves. A common canal for these two cranial nerves is also found in the 6.6-mm. *Gasterosteus*, so it may be stated that *Syngnathus* is more precocious in development in this part of the cranium than in the more anterior parts, since all of the parts so far noted have been comparable in state of development to the same parts in the 5.7-mm. *Gasterosteus*.

The occipital masses lateral to the notochord persist for a short distance posteriorly, but do not meet dorsal to the brain to form an occipital arch (figs. 2, 8). They gradually diminish in extent, the ventrolateral parts disappearing first, as would be expected, since these represent the posterior ends of the parachordals. Finally, the cartilage on the dorsolateral surfaces

of the notochord is replaced by densely cellular masses of fibrous connective tissue. There are no postvagial nerves in this region and the first neural arch is relatively far distant from the posterior end of the occipital cartilages.

The postorbital process of the otic capsule appears in the posterior part of the membranous orbital wall (fig. 5), and its anterior end does not extend as far dorsal in the wall as does its homologue in a 6.6-mm. *Gasterosteus*. In cross-section the postorbital process is very thin and flat, increasing in thickness posteriorly. At the same time it trends ventrally in the cranial wall (fig. 1). The jugular vein and the gasserian ganglion lie ventral to the postorbital process, and for this reason Swinnerton compared the postorbital process of *Gasterosteus* to a part of the alisphenoid cartilage of *Salmo* (Parker, '72)—a comparison with which I am in full agreement as regards the postorbital process of *Syngnathus*, both from this relation and also from its connection with the otic capsule.

Posteriorly, the dorsal end of the postorbital process becomes bulbous, the abductor hyomandibularis muscle having its origin on the lateral face. Posterior to the origin of this muscle the dorsal end of the hyomandibula articulates in a groove on the lateral face of the postorbital process (fig. 6). As noted above, the ventral end of the postorbital cartilage becomes the prootic process which trends ventrally and meets the trabecula cranii (figs. 1, 2).

The ramus hyomandibularis facialis and the jugular vein leave the cranium posterior to the prootic process, the dorsal margin of the foramen being formed by the postorbital process (fig. 6). The adductor hyomandibularis muscle has its origin on the ventroposterior margin of this foramen. Similar topographical relationships between the cartilage and the cranial nerves have been described for *Gasterosteus*, but the muscle relations have not been noted. In a 25-mm. *Salmo* (Gaupp), a band of cartilage (präfaciale basicapsuläre Commissure) connects the postorbital process with the parachordal and is perforated by three foramina—an anterior one for the jugular vein, a posterior one for the ramus hyomandibularis facialis, and a ventral one for the ramus palatinus facialis.

In *Syngnathus* the cartilage forming the dorsal margin of the foramen for the passage of the jugular vein and the ramus hyomandibularis facialis extends more dorsally in the wall than does the postorbital process (fig. 1). Posterior to the foramen the cartilage forms the wall of the otic capsule proper, since the membranous labyrinth appears between its wall and the brain (fig. 7). The large basicapsular fenestra in the wall of the otic capsule of a 5.7-mm. *Gasterosteus* and of a 13-mm. *Salmo* has been interpreted as the homologue of the fenestra ovalis in the otic capsule of *Amphibia*. This is represented in the wall of the otic capsule of *Syngnathus* by a minute opening in its ventral part, closed by membrane.

Two cartilaginous projections from the mesial face of the capsular wall, connected by fibrous tissue with the membrane separating the cavum cranii from the cavum labyrinthii, are the primordia of the lateral and posterior septa semicircularia. The lateral septum is shown in figure 7. The posterior one is located a short distance posterior to this and more dorsal.

The dorsomesial margins of the otic capsular walls are continuous with a narrow median cartilage which lies in the fibrous connective tissue dorsal to the brain (fig. 2). This bar of cartilage starts as a small point in the membranous roof of the cranium of the postorbital region in the same transverse plane as that in which the facialis foramen is located (fig. 6). It gradually becomes wider posteriorly and forms a triangular plate in the cranial roof (fig. 2). The posterolateral margins of this plate are confluent laterally with the mesial margins of the otic capsular walls. Such a plate of cartilage has been described, but not figured for a 25-mm. *Salmo* by Gaupp, and termed the *taenia tectum medialis*. An epiphysial bar such as is found in the cranial roof of *Gasterosteus* connected with the ectethmoid region by a pair of supraorbital cartilages is lacking in *Syngnathus*. Hence in *Syngnathus* the *taenia tectum medialis* is the only cartilage which at this stage lies dorsal to the brain, since the occipital cartilages have not as yet met mesially. It may be considered as the remnant of a once solid cartilaginous synotic tectum which has become very much reduced during the phylogenetic processes which gave rise to *Syngnathus*.

As already mentioned, the posterior wall of the otic capsules becomes confluent with the occipital processes and forms a canal on either side of the chondrocranium for the passage of the glossopharyngeal and vagus nerves.

### *B. The visceral arches*

The first part of the primordial visceral skeleton to be considered is a small median precranial cartilage, the rostral cartilage (figs. 1, 2, 3). Lying on the middorsal surface of the rostral process of the ethmoid plate, it has a relationship to the latter, comparable to that in other teleosts, as mentioned by Gaupp. This cartilage was not noted by McMurrich in *Syngnathus peckianus*. Sagemehl ('91), in considering the rostral cartilage in other teleosts, has homologized it to the median synchondrosis which occurs between the distal ends of the palatoquadrate cartilages of *Heptanchus*. This homology is further borne out by the conditions in *Syngnathus* at this stage, because the rostral cartilage is connected by densely cellular connective tissue with the anterior ends of the palatine cartilages. The beginnings of the premaxillary ossifications are connected with the lateral surface of this rostral cartilage (fig. 3). It has no homologue in *Gasterosteus*.

The anterior ends of the palatine cartilages (ethmopalatines, McMurrich) are flat in cross-section, the mesial surface of each articulates with the lateral surface of the rostral process of the ethmoid cartilage and is connected by fibrous connective tissue with the latter and with the rostral cartilage (figs. 1, 3). Posterior to the region of articulation, each palatine cartilage tapers and trends ventrally, finally dwindling to a small point embedded in the embryonal connective tissue surrounding the ethmoid plate and the olfactory pit (fig. 1). It is important to note here that the palatine cartilage has a posterior fibrous connection with the dorso-anterior margin of the pterygoquadrate, rather than a cartilaginous connection as in *Salmo* or *Gasterosteus*. This condition in *Syngnathus* bears out the statement made by Swinnerton to the effect that the palatine cartilage does not arise

independently in any teleost, but must at least have a fibrous, if not a cartilaginous connection with the more posteriorly situated pterygoquadrate. Such a condition exists at this stage in *Syngnathus*, and whatever the later conditions may be, this fibrous connection is primary. In this respect the relations of the posterior end of the palatine cartilage resemble that of the 10-mm. *Amiurus* (Kindred) in which the posterior end of the palatine is connected with the anterior end of the pterygoquadrate by a connective-tissue bridge.

McMurrich in his description of *Syngnathus peckianus* failed to recognize this relationship and stated that the 'ethmopalatine' was independent of the pterygoquadrate. The anterior relation of the palatine cartilage to the ethmoid plate is similar to that of *Gasterosteus*—a condition which Swinnerton calls *acrartete*. If it is assumed that the fibrous connection is the homologue of the intervening cartilage in *Salmo*, then the fibrous connection between the posterior end of the palatine and the anterior end of the pterygoquadrate which passes ventral to the ectethmoid cartilage in *Syngnathus* may represent a condition comparable to that in *Salmo*, where the posterior part of the palatine process of the palatoquadrate articulates with the ventral surface of the ectethmoid process.

In the 8-mm. *Syngnathus*, the mandible is formed by the fused meckelian cartilages. They project for a short distance beyond the anterior margin of the dorsal part of the oral gape and form the axes of the shovel-like ventral portion (figs. 1, 2). The anterior end of each meckelian cartilage abuts against its fellow in the median line by a flat thickened surface. The cartilage cells on the abutting surfaces are very small, numerous, and arranged in a vertical row, separated from each other by the fused perichondria. Posteriorly, the cartilages diverge, become smaller in cross-section, and are connected with each other mesially for a short distance by a slender band of developing muscle tissue. The muscle tissue is replaced more posteriorly by embryonal connective tissue. Each meckelian cartilage is gradually compressed to form the coronoid process (fig. 1). A small notch on the anteroventral face of this region separates

off an angular process, just posterior to which the cartilage articulates with a groove on the dorsal surface of the quadrate portion of the pterygoquadrate.

Meckel's cartilage of the 8-mm. *Syngnathus* differs in several respects from that of the 6.6-mm. *Gasterosteus*. In the first place, the curve of the ventral surface of this cartilage in *Syngnathus* is concave, while in *Gasterosteus* it is convex. There is a continuous gradation from the anterior part of the cartilage into the coronoid process in the former, while in the latter, the coronoid process projects abruptly from the dorsal surface. In *Syngnathus* the posterior end of the cartilage projects dorsal to the quadrate—a condition not found in *Gasterosteus*. Of the angular notch and process of *Syngnathus*, the latter only is present in *Gasterosteus*.

A rudimentary inferior labial cartilage is represented by a cellular mass which extends along the dorsal surface of the anterior part of Meckel's cartilage and ends posteriorly in the mandibular fold. It is connected by a bridge of connective-tissue cells with the primordium of the maxillary bone which lies in the supramandibular fold connected dorsally with the lateral surface of the palatine cartilage by fibrous connective tissue.

The quadrate portion of the pterygoquadrate cartilage starts anteriorly between the posterior end of Meckel's cartilage and the distal end of the symplectic (fig. 1). A narrow bridge of cartilage connects it with the latter element, showing possibly a common origin for the cartilage in this region. In cross-section the quadrate is dumb-bell shaped and lies at right angles to the articular surface of Meckel's cartilage. The posterior part of it projects dorsally as a flattened plate, connected with the posterior end of the palatine cartilage by a densely cellular strand of fibrous connective tissue and separated from the ethmoid cartilage by a fold of the oral cavity (fig. 4). The symplectic cartilage extends along the ventromesial surface of the pterygoquadrate. This cartilage ends posteriorly in the midregion of the orbit with a small posteriorly projecting process—the metapterygoid process (fig. 1). According to Swin-

nerton, the metapterygoid process of *Syngnathus* represents a stage in the reduction of an elongate metapterygoid process such as is found in other teleosts. The metapterygoid process does not have the intimate relation with hyomandibula which characterizes the metapterygoid process of *Salmo* and *Amiurus*.

As already stated, the symplectic element extends ventral to the anterior margin of the pterygoquadrate and is confluent with it by means of a bridge of cartilage. Continuing posteriorly as a slender cartilaginous core, the symplectic extends mesial to the pterygoquadrate along the entire extent of the latter (figs. 1, 2). A ventral diverticulum of the oral cavity separates it from the copula communis posterior to the pterygoquadrate. Histologically, it has a very heavy perichondrium and there are usually two or three cartilage cells in a cross-section. Posterior to the orbit, the symplectic is connected to the trabecula communis by several strands of embryonic muscle tissue. Finally it becomes confluent with the ventral end of the hyomandibula, no line of division being present between the two. A similar condition is met in the symplectic of *Gasterosteus*, but as yet the cartilaginous continuity between the distal end of the symplectic and the pterygoquadrate characteristic of *Syngnathus* has not been described. The great distance between the metapterygoid process of the pterygoquadrate and the symplectic is to be noted in *Syngnathus* as compared to the intimate relation between the elongate metapterygoid and the symplectic in *Gasterosteus* and *Belone* (Swinerton).

The hyomandibula at this stage is a rectangular piece of cartilage which articulates at its dorsal end with the anterior fourth of the otic capsule and is confluent ventrally with the symplectic (fig. 1). Ventromesially, it is flattened for articulation with the stylohyal cartilage (fig. 6). The dorsal end of the hyomandibula is thin and rounded where it abuts against the otic capsular wall. The ventral portion is thickened (fig. 6). Near the anterior dorsal margin a small foramen is present for the passage of the ramus hyomandibularis facialis. The abductor hyomandibularis muscle is inserted on the anterior margin of this foramen. The opercular process projects from the posterior

margin of the dorsal part of the hyomandibula and the adductor hyomandibularis muscle is inserted on its mesial face.

Here again certain differences are to be noted between the chondrocranium of *Syngnathus* and that of a 5.7-mm. *Gasterosteus*. The hyomandibula of the former is more elongate and rectangular on its vertical axis than is the hyomandibula of the latter. The relative amount of articular surface with the otic capsule is greater in *Gasterosteus* than in *Syngnathus*. The ventral portion of the hyomandibula of *Syngnathus* is thicker

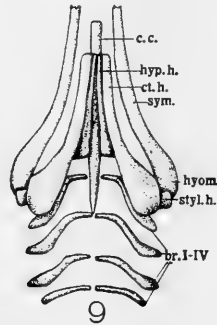


Fig. 9 Ventral view of hyoid and branchial arches, 8-mm. *Syngnathus*. Drawing made from wax model 230 times actual size of parts. Ratio of drawing to model, 1:3.

#### ABBREVIATIONS

*br.I-IV*, branchial arches I to IV  
*c.c.*, copula communis  
*ct.h.*, ceratohyal cartilage  
*hyom.*, hyomandibula

*hyp.h.*, hypohyal cartilage  
*styl.h.*, stylohyal cartilage  
*sym.*, symplectic cartilage

than the dorsal part, while in *Gasterosteus* the conditions are reversed. The distinct opercular process present in *Syngnathus* is lacking in *Gasterosteus*. The foramen for the passage of the ramus hyomandibularis facialis is the only constant feature of the body of the hyomandibula of these two forms.

The copula communis is at this stage a delicate cylindrical bar of cartilage which forms the median articulating support for the hypohyal cartilages and the first and second branchial arches (fig. 1, 9). It is supported in the embryonal connective tissue ventral to the oral cavity (fig. 4). The cartilage is continuous and of uniform caliber in its anterior part, tapering posteriorly



as a core of procartilage cells, against which abut the third and fourth branchial arches.

The copula communis of a 5.7-mm. *Gasterosteus* has approximately the same relations to the hypohyals and the first and second branchial arches, but in addition it has extended as cartilage as far as the third branchial arch. There is also a small separate cartilage between the fourth pair of branchials. The independent wedge-shaped piece at the anterior end of the copula communis of *Gasterosteus* is lacking in *Syngnathus*.

The branchial cartilages of *Syngnathus* have not curved dorsally at their distal ends in this stage as they have in the 5.7-mm. *Gasterosteus*, nor has the fifth branchial arch appeared. The pharyngobranchial plates present in *Gasterosteus* are represented in *Syngnathus* by a pair of procartilaginous masses ventral to the parachordals and connected with each other by a sheet of muscle.

The hyoid elements are very well developed in *Syngnathus*. The stylohyal is a small broad plate between the ventral end of the hyomandibula and the dorsolateral surface of the ceratohyal (fig. 9). This element, the stylohyal, lies closely ventral to the posterior margin of the hyomandibula, and not ventral to its anterior margin as does the corresponding element in *Gasterosteus*.

The ceratohyal is very massive at this stage and extends anteriorly rather than directly mesial, as in *Gasterosteus*. It abuts against the ventrolateral margin of the elongate, horizontal hypohyal. This latter cartilage is very peculiar in its relation and extent. Instead of being a small wedge-shaped articular plate, as in *Gasterosteus*, it is an elongate rod of cartilage which anteriorly extends beyond the end of the ceratohyal (fig. 9). The surface of articulation between the hypohyal and the copula communis is nearer to the posterior end of the hypohyal than to its anterior end.

## THE CHONDROCRANIUM OF THE 12-MM. STAGE

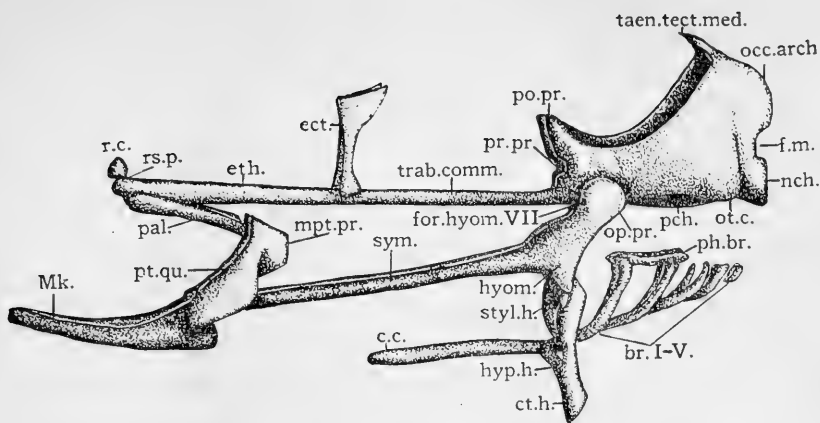
*A. The neurocranium*

The anterior end of the ethmoid plate of the 12-mm. *Syngnathus* is much broader and blunter than it is in the neurocranium of the 8-mm. stage. Its relations to the palatine and rostral cartilages are similar (fig. 11). The anterior end, however, is no longer turned dorsally, but has straightened out, carrying with it the attached elements. Posterior to the broad rostral process, the ethmoid plate gradually diminishes in size. It becomes wedge-shaped at first and then more triangular in cross-section. The dorsal part of the ethmoid is the thicker.

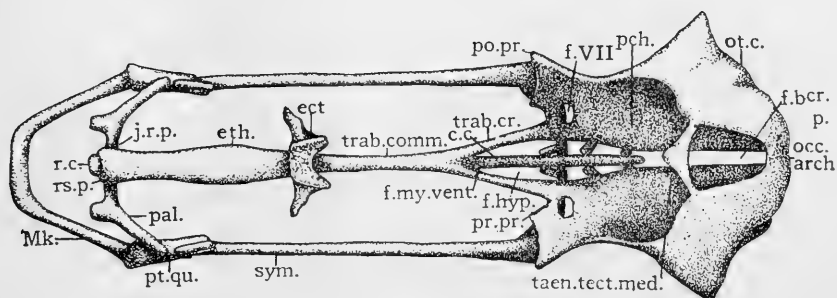
The vomer primordium represented in the 8-mm. stage by a mass of cells ventral to the ethmoid plate is now an osseous lamella embedded in a mass of osteogenetic cells in the same region (fig. 12).

The dorsal surface of the ethmoid plate is surmounted by a septum of fibrous-connective tissue. The relationship of this to the cartilage is the same as that of the osseous ridge which later develops in this place. This ridge of connective tissue is connected with the stroma enclosing the olfactory pits and is continuous posteriorly with the membranous interorbital septum. Histologically, the ethmoid cartilage is the same as it is in the 8-mm. stage except for an increase in size and the presence of a thicker perichondrium.

The ectethmoid cartilages, which in the 8-mm. embryo were separate, connected with each other by connective tissue only, have now fused with each other dorsomesially to form a horse-shoe-shaped mass of cartilage posterior to the olfactory pits (fig. 12). The posterior margin of this mass forms the anterior boundary of the orbits. The anterior end of the brain lies in a trough on the dorsum of this ectethmoid arch, the margins of the trough projecting posteriorly for a short distance in the membranous cranial wall (figs. 10, 11). The olfactory nerves, after leaving the olfactory pits, pass to the mesial margins of the sides of the arch and extend posteriorly within it (fig. 12). Thus at this stage the olfactory nerves have not been separated from



10



11

Fig. 10 Lateral view of the chondrocranium and visceral arches of a larva of *Syngnathus fuscus*, 12 mm. long. Drawing made from wax model 150 times the actual size of the chondrocranium. Ratio of drawing to model, 1:3.

Fig. 11 Dorsal view of the same model as shown in figure 10.

#### ABBREVIATIONS

*br.I-V*, branchial arches I to V.  
*c.c.*, copula communis  
*ct.h.*, ceratohyal cartilage  
*ect.*, ectethmoid cartilage  
*eth.*, ethmoid plate  
*f.bcr.p.*, fenestra basiscranii posterius  
*f.hyp.*, fenestra hypophyseos  
*f.my.vent.*, fenestra myodomeus ventralis  
*f.m.*, foramen magnum  
*f.VII*, foramen for exit of ramus hyomandibularis facialis  
*For.hyom.VII*, foramen in hyomandibula for ramus hyom.fac.  
*hyom.*, hyomandibula  
*hyp.h.*, hypohyal cartilage  
*j.r.p.*, junction rostro-palatinus  
*Mk.*, Meckel's cartilage

*mpt.pr.*, metapterygoid process  
*nch.*, notochord  
*occ.arch.*, occipital arch  
*ot.c.*, otic capsule  
*pal.*, palatine cartilage  
*p.ch.*, paracordal cartilage  
*ph.br.*, pharyngobranchial cartilage  
*pt.qu.*, pterygoquadrate cartilage  
*po.pr.*, postorbital process  
*pr.pr.*, prootic process  
*r.c.*, rostral cartilage  
*rs.p.*, rostral process of the ethmoid  
*styl.h.*, stylohyal cartilage  
*sym.*, symplectic cartilage  
*taen.tect.med.*, taenia tectum medialis  
*trab.comm.*, trabecula communis  
*trab.cr.*, trabecula cranii

each other by cartilage. They enter the brain at the posterior margin of the mesial dorsal part of the arch. It is significant to note here that the olfactory nerves are in the process of being passively enclosed in cartilage and do not actively fenestrate it.

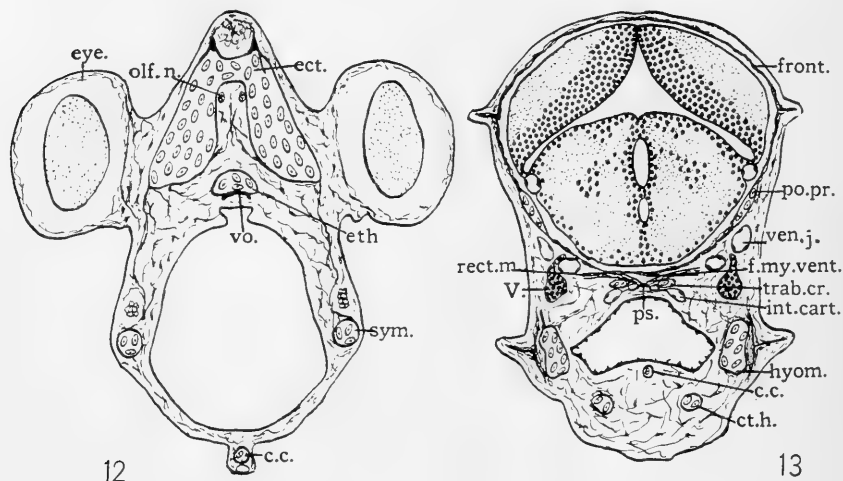


Fig. 12 Cross-section through the ectethmoid region, 12-mm. *Syngnathus*. Semidiagrammatic. Camera lucida.  $\times 102$ .

Fig. 13 Cross-section through the posterior end of the orbit, 12-mm. *Syngnathus*. Semidiagrammatic. Camera lucida.  $\times 66$ .

#### ABBREVIATIONS

<i>c.c.</i> , copula communis	<i>olf.n.</i> , olfactory nerve
<i>ct.h.</i> , ceratohyal cartilage	<i>po.pr.</i> , postorbital process
<i>ect.</i> , ectethmoid cartilage	<i>ps.</i> , parasphenoid ossification
<i>eth.</i> , ethmoid plate	<i>rect.m.</i> , recti eye muscles
<i>f.my.vent.</i> , fenestra myodome ventralis	<i>sym.</i> , symplectic cartilage
<i>front.</i> , frontal ossification	<i>trab.cr.</i> , trabecula cranii
<i>hyom.</i> , hyomandibula	<i>V</i> , ganglion of trigeminal nerve
<i>int.cart.</i> , internal carotid artery	<i>ven.j.</i> , vena jugularis
	<i>vo.</i> , vomer ossification

The ethmoid plate lies ventromesial to the arch, connected with it by the fibrous connective-tissue ridge already mentioned, as extending along the dorsal surface of the ethmoid. In *Salmo*, *Gasterosteus*, and *Amiurus*, the ectethmoid processes are outgrowths from the ethmoid cartilage. If the ectethmoid carti-

lages of *Syngnathus* are to be regarded as remnants of the olfactory capsules, the condition of the ectethmoids in the above forms indicates a more specialized relation. Therefore, I am regarding the relation of the ectethmoid cartilages of *Syngnathus* as primitive.

There is no tegmen or cartilage dorsal to the brain in the ethmoid region, the tips of the frontal ossifications appearing as a pair of delicately spined osseous lamellae in the fibrous connective tissue enclosing the brain.

Just posterior to the ectethmoid region the oblique eye muscles have their origin in the fibrous tissue of the interorbital septum and are not related to the cartilage of either the ethmoid plate or the ectethmoid cartilages. The trabecula communis is flatter and broader in cross-section than that part of the ethmoid plate immediately ventral to the ectethmoid arch, but it gradually diminishes, until at the anterior margin of the fenestra myodorus ventralis it is a very slender cylindrical bar. The parasphenoid lamella is attached to its ventral surface by connective tissue.

In the region of the anterior margin of the fenestra myodorus ventralis, the internal carotid arteries, which more anteriorly were dorsal to the trabecula communis, now pass ventrally into the space between the trabeculae cranii (fig. 13). The recti eye muscles enter the fenestra myodorus ventralis dorsal to the internal carotid arteries between the trabeculae cranii and the fibrous connective tissue enclosing the brain. The hypophysis cerebri lies posterior to this region, so that, in accordance with Allis' criteria, this space between the anterior ends of the trabeculae cranii has been termed the fenestra myodorus ventralis, and not the fenestra hypophyseos. As in the younger stage, the parasphenoid ossification forms the floor of the fenestra myodorus ventralis and serves as a surface of attachment for the recti muscles. The anterior end of the hypophysis cerebri appears just posterior to this region, and the space occupied more anteriorly by the recti muscles and by the carotids is obliterated, the hypophysis pushing the meningeal tissue against the trabeculae cranii. This portion of the intertrabecular fenestra may be properly termed the fenestra hypophyseos.

As in the younger stage the prootic processes abut against the lateral surfaces of the trabeculae and are separated from them by their perichondria (figs. 10, 11). Except for a greater size of all parts concerned, the foramen for the passage of the ramus hyomandibularis facialis and the jugular vein bears the same relation to the trabeculae and prootic processes as in the 8-mm. stage. In this region the trabeculae pass insensibly into the parachordals. These immediately begin to thicken and are fused laterally with the ventromesial walls of the otic capsules (fig. 11). As the hypophysis tapers posteriorly the fenestra hypophyseos begins to narrow and the parasphenoid lamella dwindles to a mere spicule of bone forming its floor. Where the hypophysis ends the anterior tip of the notochord appears between the parachordals. It is enclosed in the fenestra basi-cranii posterius. The roof of this fenestra is formed by fibrous connective tissue confluent on each side with the dorsal perichondria of the parachordals. The floor is formed by fibrous connective tissue confluent with the ventral surface of the parachordals. The notochord is free and is not enclosed in cartilage as is the intercranial notochord of the 6.6-mm. *Gasterosteus*. The notochord is, however, much more closely applied to the cartilage in *Syngnathus* than is the notochord of a 19-mm. *Amia*.

The parachordal-occipital process fusion in conjunction with the posterior wall of the otic capsule forms as in the 8-mm. stage, the canal for the passage of the glossopharyngeal and vagus nerves. Ossification has not begun in this region.

The postorbital process bears the same relation to the trigeminal ganglion that it did in the younger stage, but a deeper notch occurs in the anterior margin of the prootic process posterior to the ganglion (figs. 11, 13). This notch is the homologue of the incisivum prooticum of *Salmo*. As in the younger *Syngnathus* the perichondria between the trabecula and the prootic process persist.

Due to the lateral growth of the brain and an increase in size of the ramus hyomandibularis facialis and the jugular vein, the foramen through which these pass is more ventral in the wall

and larger than it is in the 8-mm. stage (fig. 11). The lamella of the frontal ossification is continuous with the perichondrium of the cartilage forming the dorsal margin of the foramen. Just posterior to the foramen, the cartilage is more deeply grooved for articulation with the hyomandibula than it is in the younger stage. The articular surface does not reach as far posteriorly as that part of the otic capsule containing the membranous labyrinth (fig. 10). The membranous labyrinth has increased greatly in size since the 8-mm. stage and consequently expanded laterally, displacing to a more ventral position the cartilage which formed the lateral wall of the otic capsule in the younger stage. A thin lamella of cartilage surmounts the margin of the thicker ventral portion and forms the dorsolateral wall of the capsule in this region. The formation of the septa semicircularia has not gone farther than in the younger stage and are as yet membranous. The small fenestra basicapsularis present in the otic capsule of the 8-mm. stage persists. Toward the posterior portion of the cranium the mesiodorsal margins are continuous with the cartilage of the taenia tectum medialis (fig. 11). The latter is enclosed in an osseous lamella which represents the beginning of the supra-occipital ossification. Some of the cartilage is in the process of resorption.

The occipital processes which in the younger stages were separate from each other have fused mesially, so that an occipital arch is formed. The posterior margin of the arch projects for a short distance beyond the sides of the arch. The fontanelle left between the taenia tectum medialis and the occipital arch is closed by membrane.

#### *B. The visceral arches*

The rostral cartilage has the same relation to the dorsal surface of the ethmoid plate that it had in the younger stage, but the premaxillary lamellae which are represented in the younger stage by cellular masses have now ossified and extend laterally from the rostral piece, overlapping the anterior ends of the palatines.

The palatine cartilages have the same relation to the rostral process of the ethmoid as before, but are longer than in the younger stage and lie in a more horizontal plane—a condition due to the straightening out of the anterior end of the ethmoid (fig. 10). Posteriorly, the palatine cartilages have grown along the fibrous strand which in the younger stage connects them with the pterygoid portion of the pterygoquadrate plates. As a result of this growth, the palatine cartilages and the pterygoquadrates are in closer proximity than they are in the younger stage (fig. 10).

The mandibular symphysis is broader and thicker than in the 8-mm. stage and the meckelian cartilages diverge from it as a pair of cartilaginous rods, which thicken gradually at their posterior ends to form the coronoid processes (fig. 10). The mandible is now more typically teleostean than it is in the younger stage, the peculiar concavity noted on the ventral surface in the 8-mm. stage has been obliterated. The primordium of the dentary ossification appears around each Meckel's cartilage as a single lamella, lateral to and separate from the cartilage. No teeth are present. As in *Gasterosteus*, the angular process projects posteriorly beyond the surface of articulation with the pterygoquadrate. The pterygoquadrate articulates with the dorsal surface of this portion of Meckel's cartilage at an oblique angle. It becomes vertical posteriorly and there is no longer a projection of the posterior end of the coronoid process dorsal to it. The symplectic element extends ventral to the posterior mesial region of the pterygoquadrate and the metapterygoid process projecting from this region is longer than it is in the 8-mm. stage (fig. 10).

Due to the straightening out of the mandible and the enlarging of the oral opening, the notch present on the ventro-anterior margin of the distal portion of each Meckel's cartilage has disappeared. This whole region has been pushed farther anterior than it is in the 8-mm. stage, by growth and elongation of the symplectic element which has grown in concert with the ethmoid cartilage. The posterior end of Meckel's cartilage no longer meets the distal end of the symplectic.



The mass of cells indicated in the 8-mm. stage as the homologue of the inferior labial cartilage of *Gasterosteus* has not changed. However, the cells which represented the center of formation of the maxillary ossification have deposited an elongate, vertical lamella. This lamella is connected dorsally with the premaxillary lamella anterior to the palatine articulation, and ventrally by a chain of cells with the dorsal tip of the dentary lamella. The maxillary lamella supports the lateral wall of the oral cavity between the end of the cranium and the palatine cartilage. The maxillary lamella bears no teeth.

The metapterygoid process is longer than it is in the 8-mm. embryo, but even now does not have a relation to the symplectic like that of the larval *Gasterosteus*. As Swinnerton states, the metapterygoid process of *Syngnathus* represents a stage in the disappearance of such a structure in the teleosts. The posterior end of this process which in the 8-mm. stage lies posterior to the transverse plane of the ectethmoid cartilages now lies quite far anterior to them (fig. 10).

The distal end of the symplectic cartilage which in the 8-mm. stage extends the whole length of pterygoquadrate now reaches only to its posterior end. The cartilaginous connection between these two elements has disappeared. The elongation of the symplectic and the subsequent changes in the positional relationships in that part of the visceral apparatus lying anterior to it have already been stated. Ossification has appeared in the form of a curved lamella external to and distinct from the perichondrium of the symplectic shaft. Posteriorly, the proximal end of the symplectic gradually widens as it becomes confluent with the hyomandibula, so that the angle, which in the younger stage appears between the proximal end of the symplectic and the anterior margin of the hyomandibula, has been obliterated. As a result, it has much the same appearance as that of the 6.6-mm. *Gasterosteus*.

The hyomandibula, instead of having the vertical position of the 8-mm. stage, is now directed anteriorly at its ventral end, so that the bulk of the cartilage lies anteroventral to the surface of articulation (fig. 10). The posterior extent of the hyoman-

dibula is not nearly as great as it is in *Gasterosteus* or *Salmo*. Although the whole ventral portion has swung anteriorly, the foramen for the passage of the ramus hyomandibularis facialis lies as before near the anterior margin of the dorsal head of the

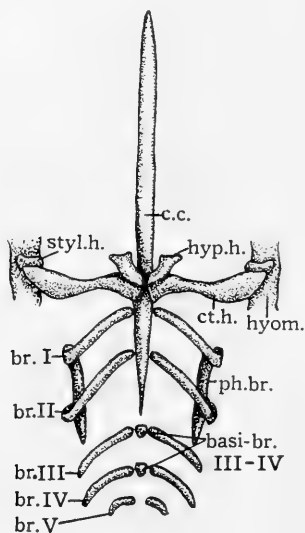


Fig. 14 Ventral view of hyoid and branchial arches, 12-mm. *Syngnathus*. Drawing made from wax model 150 times actual size. Ratio of drawing to model, 2:3.

#### ABBREVIATIONS

<i>basi-br. III-IV</i> , basibranchial cartilages III and IV	<i>hyom.</i> , hyomandibula
<i>br. I-V</i> , branchial arches I to V	<i>hyp.h.</i> , hypohyal cartilage
<i>c.c.</i> , copula communis	<i>ph.br.</i> , pharyngobranchial cartilage
<i>ct.h.</i> , ceratohyal cartilage	<i>styl.h.</i> , stylohyal cartilage

hyomandibula. As in the 8-mm. stage, the dorsal portion is relatively thinner than the ventral.

The mesioventral surface of the hyomandibula is flattened for articulation with the interpolated stylohyal, the plane of articulation having been carried anteriorly by the shift in the axis of the hyomandibula (figs. 10, 14). The shift which has taken place in the hyomandibula is due to the part played by the latter in changing the size of the oral cavity in the process

of respiration, since *Syngnathus* at this stage is a free-swimming larva. An osseous lamella lies external to and separate from the ventral portion of the hyomandibula, enclosing a blood-vessel between it and the perichondrium (fig. 13).

As in the 8-mm. stage, the stylohyal element is interpolated between the dorsal end of the ceratohyal and the hyomandibula (fig. 14). The ceratohyal, which in the same stage (when the embryo is yet enclosed in the brood pouch of the parent) extends anteroposteriorly in an almost horizontal plane with the posterior end in the more ventral position, has now changed in position, so that it lies almost vertical (fig. 10). The dorsal end of each ceratohyal articulates with the stylohyal of that side, while the ventral end, formerly parallel with the hypohyal element, now lies ventroposterior to it.

The hypohyal element has also undergone a change in position. Instead of lying in a horizontal plane parallel to the *copula communis*, it now occupies a vertical position, articulating on its mesiodorsal surface with the *copula communis* (fig. 14). The change in the positional relationships of these elements is due to the functional activity of these as the supporting cartilages of the oral cavity, which by its changes in shape draws water into the mouth and expels it over the gills. Since the gills were not developed in the 8-mm. stage, these parts had not shifted. The conditions of these elements in the 12-mm. stage of *Syngnathus* are more nearly like those of the 6.6-mm. *Gasterosteus*, which indicates an earlier functional activity of these parts in the latter, correlated with the longer protected period of *Syngnathus*.

The *copula communis* of the 12-mm. stage has retained the same relative length as before, but is more widely separated from the ventral surface of the cranium, because of the increase in the size of the oral cavity (figs. 10, 14). Posteriorly it extends beyond the second branchial arch after having been displaced ventrally between the hypohyal elements due to the shift in their position. Its posterior end is connected by a cord of procartilaginous cells with the independent basibranchial cartilages between the third and fourth branchial arches.

The first branchial arch has elongated and is turned up laterally to fuse with the pharyngobranchial cartilage which lies at the laterodorsal margin of the oral cavity (figs. 13, 14). The pharyngobranchial plate is also connected with the lateral end of the second branchial arch, ending just posterior to it (fig. 14). The third and fourth branchial arches have not as yet turned up laterally, but they are longer than they are in the 8-mm. stage. The fifth branchial arch has appeared as a pair of small cartilages lateral to the procartilage continuum of the basi-branchial plate of the fourth arch (fig. 14).

#### SUMMARY AND CONCLUSIONS

Under the classification of the teleosts by Gregory ('07), *Syngnathus* is placed in the order Lophobranchii, which with the order Hemibranchii (including *Gasterosteus*), are grouped into a superorder, the Thoracostraci. The important similarities and differences of the chondrocrania of these two forms are stated in the following summary.

Both *Gasterosteus* and *Syngnathus* are alike in the following chondrocranial characters: the presence of an elongate ethmoid region; the acartete articulation of the palatine cartilages; the incomplete cranial roof; the horizontal position of the trabeculae cranii and the parachordal cartilages; the presence of two septa semicircularia in each otic capsule; a prootic process separating the trigeminal and facialis nerves from each other; an elongate fenestra basicranii posterius; a common foramen for the glossopharyngeal and vagus nerves between the otic capsule and the occipital arch; a small pterygoquadrate cartilage; a large intercranial notochord; a postorbital process at the anterior margin of each otic capsule.

The above characters wherein *Syngnathus* resembles *Gasterosteus* indicate the direction in which the skulls of these two forms have become specialized. In addition, further specializations are to be noted in the chondrocranium of *Syngnathus*:

1. The modification of the anterior end of the ethmoid plate by turning dorsally in the 8-mm. stage.

2. The presence of a definitive fenestra myodome ventralis between the anterior ends of the trabeculae cranii.

3. The absence of cartilage on the dorsal and ventral surfaces of the intercranial notochord.

4. The presence of a taenia tectum medialis in the cranial roof.

5. The presence of a minute basicapsular fenestra in the ventral wall of each otic capsule.

6. The fibrous connection between the posterior end of the palatine cartilage and the dorsal end of the pterygoquadrate cartilage.

7. The presence of an elongate symplectic cartilage and a slender hyomandibula.

8. The great length of the trabecula communis and the ethmoid plate.

9. The change in position of the hyoid elements during development.

10. The presence of a reduced metapterygoid process.

The chondrocranial characters of *Syngnathus* which may be regarded as primitive are:

1. The presence of a rostral cartilage which has been considered by Sagemehl in other teleosts as the homologue of the median synchondrosis between the palatopterygoid cartilages of *Heptanchus*.

2. The development of the ectethmoid cartilages independently of the ethmoid plate, which seems to the author to indicate that they are the remnants of an olfactory capsule which developed independently.

3. The open communication between the cavum labyrinthii and the cavum cranii.

4. The absence of postvagal nerves in the occipital region.

From the above preponderance of specialized characters, it may be concluded that even in its early stages of development the skull of *Syngnathus* is already highly specialized.

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El comportamiento genético de los cromosomas heteromórficos  
homólogos de *Circotettix* (Ortópteros).

*Circotettix verruculatus* posee tres pares de cromosomas claramente diferenciables, y cuyos miembros pueden ser telomíticos o atelomíticos. Un análisis citológico de cuarenta machos salvajes ha suministrado la frecuencia relativa de la aparición de los dos tipos de cromosomas homólogos para cada uno de los tres pares. En 28 descendientes machos procedentes de cinco cruzamientos, en los cuales se conocían los complejos cromosómicos de los padres, se presentan 56 homólogos ( $2 \times 28$ ) para cada uno de los tres pares, o sea un total de 168 homólogos ( $3 \times 56$ ) en todos ellos, que podrían haber variado. Han debido tener lugar 22 cambios morfológicos en dicho número de homólogos si existe una reorganización durante la ontogenia que repita las condiciones de la especie.

Por el contrario, ni uno solo de los descendientes difiere en su constitución cromosómica más allá de los límites que deben anticiparse al existir una combinación de los gametos de sus padres. Las razones de combinación de los dos tipos de homólogos en cada uno de los tres pares son las que resultarían de la unión al azar de los gametos de los padres. De este modo, mediante identificación actual de los homólogos de un par dado de cromosomas desde los padres a los descendientes y con la determinación de sus relaciones de recombinación, el paralelismo entre el comportamiento de los cromosomas y los fenómenos mendelianos es completo.

Translation by José F. Nonidez  
Cornell Medical College, New York



# GENETICAL BEHAVIOR OF HETEROMORPHIC HOMOLOGOUS CHROMOSOMES OF CIRCOTETTIX (ORTHOPTERA)

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FIVE PLATES (THIRTY-FIVE FIGURES)

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## I. INTRODUCTION

"Science begins with naïve, often mystic conceptions of its problems. It reaches its goal whenever it can replace its early" guessing by verifiable hypotheses and predictable results, Morgan ('16). The facts herein presented have been so thoroughly *guessed* as to cause almost a feeling of apology for presenting them. Since Mendel's laws became generally known in 1900 so many points have been brought out showing that the behavior of the chromosomes during maturation and fertilization pro-

vides a mechanism for carrying these laws into effect that the number of scientific people who question the proposition that the chromosomes are the bearers of the hereditary factors has become almost negligible.

Mendel's first law in substance is, that of a pair of contrasting unit factors contributed by the male and female parents, respectively, one will dominate the other in the first generation, but that during the gametogenesis of these individuals each member of a pair of factors segregates into a different germ cell. This is known as the law of segregation.

Mendel's second law is that when a number of pairs of unit factors are present the pairs assort independently of each other. That is, while the first law describes the behavior of the individuals of a pair, the second applies to the relation of the pairs to each other. This is sometimes called the law of independent assortment of different pairs of unit factors.

Mendel, of course, was dealing with unit characters from a purely genetical standpoint. Shortly afterwards, the work of the early cytologists led to the Roux-Weismann theory of heredity, which recognizes the chromosomes as the bearers of the hereditary factors. Van Beneden in 1883 reported that egg and sperm contribute equal amounts of chromatin to the new individual in *Ascaris megalocephala*. Sutton in 1902 first clearly showed that the chromosomes occur in a duplex size series and pointed out that the behavior of the chromosomes during maturation and fertilization is such as would be necessary for carrying out the laws of heredity discovered by Mendel.<sup>1</sup>

For a demonstration, however, two things were necessary: First, to find some species in which homologous chromosomes (those which unite in synapsis) differ from each other and from the remainder of the complex in such a way that they can be certainly identified. Second, to be able to breed the organisms freely in captivity in order that the behavior of the unlike homologues might be studied in both parents and offspring.

<sup>1</sup> This paper is intended to be taken in connection with the one published in 1917, deciding as it does questions left open at that time. For this reason it is not as complete in itself as it might otherwise be desirable to make it.

The first of these conditions has already been satisfactorily met; in a former paper (Carothers, '17), based on a study of the germ cells of several species of a certain group of short-horned grasshoppers, the following facts were established:

1. Given tetrads (seven out of eleven in *Trimerotropis fallax*) may be composed of morphologically dissimilar homologues.

2. When heteromorphic, the members of these pairs segregate during the first maturation division. This behavior parallels that of unit characters as established by Mendel's first law.

3. The homologues of three of these pairs were traced and found to segregate at random in regard to the accessory chromosome and consequently in relation to each other, and presumably to the members of other pairs, thus furnishing a physical mechanism such as would be necessary for the carrying out of Mendel's second law.

4. The chromosomal constitution of approximately one hundred wild individuals was such as would be expected from a free union of gametes bearing these morphologically unlike homologues.

The logical conclusion was that here, with almost diagrammatical clearness, we could trace the segregation of given homologues to the gametes and their recombination in the zygotes.

At the same time, however, the alternative possibility was pointed out that in these species there might be a reorganization at the time of fertilization which would result in a shift in point of fiber attachment and a corresponding change in the morphology of the chromosomes, such that the offspring of a single pair would tend to give the range of variation of the species instead of the range possible from a combination of the gametes of their parents. The possibility that there might be a reorganization was rendered more probable since at about the same time McClung ('17) encountered relations between the octad and hexad multiples of *Hesperotettix viridis* which seemed to indicate a reorganization of the chromosomal combination involved in the multiples at the time of fertilization. The two cases, as Dr. McClung pointed out, are not exactly comparable, since in *H. viridis* non-homologous chromosomes are involved and the mechanism may

very well be different. The solution in both instances could only be obtained by a comparative analysis of the chromosomal constitution of given pairs of grasshoppers and of their offspring. In any case, in order to complete the parallelism between the behavior of the chromosomes and Mendelian phenomena, it was necessary to trace the behavior of the heteromorphic chromosomes from parents to offspring.

## II. MATERIAL AND METHODS

Since all of the species used in my 1917 work occur only in the western half of the United States, the hope was then expressed that *Circotettix verruculatus*, whose range extends to the eastern part of the country, might be equally favorable. This expectation was justified, and combined cytological and genetical work was undertaken on this species. The stock was obtained from near Manchester, New Hampshire, July 29, 1918. Collecting during the last week of July, one obtains both adults and nymphs.<sup>2</sup>

It is necessary to obtain the females as nymphs to insure their being virgin. Males and females were kept in separate cages until about September 1, when individual matings were made up. After eggs had been laid both parents were killed and the gonads fixed; the testes in strong Flemming, the ovaries in picro-formol-acetic.

Of eighteen matings, one or the other of the parents died in six, and these cages were discarded; four of the twelve remaining cages contained no eggs, due perhaps to the exhaustion of the ovaries of the females before the matings were made up (some of the females had laid at least ten days previously). The remaining eight matings gave 138 offspring. Fifty-one was the largest number obtained from one pair. Eggs from three pods hatched from this mating.

Twenty-eight male offspring from five of the matings have been studied cytologically, and it is believed that they furnish sufficient evidence as to the point under consideration.

<sup>2</sup> It is intended to give details of the rearing and postembryonic development of *C. verruculatus* in a separate paper.

## III. OBSERVATIONS

The principal object of this paper is not to trace the spermatogenesis of *Circotettix verruculatus*, but to show as graphically as possible the genetic relationship of the chromosomes of the male and female involved in particular matings to those of their offspring. The plates accordingly are made up with the first spermatocyte chromosomes used as the standard, the spermatogonial complex and the somatic complexes of the females being rearranged so that supposedly homologous chromosomes<sup>3</sup> fall in vertical rows. A general knowledge, however, of the range of chromosomal conditions which, from the frequency of their occurrence in wild individuals, may be considered normal for the species is essential to a proper study of the specific matings. The following description based on a study of sixty-eight wild individuals is typical for members of the species from two localities, Manchester, New Hampshire, and Pigeon Cove, Massachusetts.

1. *Description of general chromosomal conditions in the species*

a. *Typical conditions.* *C. verruculatus*, like all of the species of the genus so far studied (unless *Trimerotropis suffusa* Scudd. be considered a *Circotettix*), has normally twenty-one chromosomes in the spermatogonia instead of twenty-three, the basic number in the short-horned grasshoppers. The spermatogonial complex (pl. 1, row 2) has constantly nine large atelomitic chromosomes (those with non-terminal fiber attachment) (nos. 6, 9, 10, 11, 12) and six telomitics (those with terminal fiber attachment) (nos. 2, 4, 5). The remaining six (nos. 1, 7, 8) may be individually of either type, but any given form is constant for a particular specimen. The diploid complex of the female is similar except for the presence of one additional accessory, giving constantly at least ten large atelomitics (pl. 1, row 3, nos. 6, 9,

<sup>3</sup> Size, morphology, and comparison with the component members of the tetrads have been the criteria used for determining homologues in the diploid complexes. In regard to the critical pairs numbers 1, 7 and 8, there can be no reasonable doubt since they are easily identified. For most of the other pairs the arrangement is only approximately correct both as regards the selection of homologues and the arrangement in the size series.

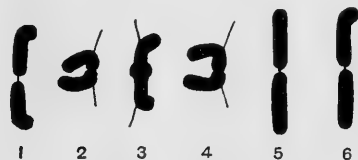
10, 11, 12). Passing to the first spermatocytes, the largest four chromosomes (pl. 1, row 1, nos. 9, 10, 11, 12) and the accessory (no. 6) are constantly atelomitic. Numbers 2, 4, and 5 are constantly telomitic, while numbers 1, 7, and 8 may vary from specimen to specimen.

Since heteromorphism is confined to these three pairs, they are the ones whose behavior in heredity is to be followed. Fortunately, they are clearly distinguishable from each other. Number 1 and number 2 are practically alike in size, but number 2 is always telomitic. Number 1, then, is the only small chromosome which may be heteromorphic or atelomitic (pl. 1, column 1). Again, number 7 and number 8 are practically indistinguishable in regard to size, but when atelomitic, number 7 has subterminal fiber attachment (pl. 1, column 7) while number 8 when atelomitic has nearly median attachment (pl. 3, column 8). In case number 8 is atelomitic, it has not been distinguished from number 9, but the essential fact that each is atelomitic is easily demonstrated.

*b. Methods of transformation of chromosome number 7; E-shaped tetrads.* As just mentioned, chromosome number 7 when atelomitic has subterminal fiber attachment. There are apparent exceptions, however, which for a time caused confusion. In practically every individual there are a few instances of this tetrad opening out in such a manner that the long instead of the short arms are free. For examples, see chromosome number 7, plate 2, row 12, and plate 5, row 33. In the latter instance it is especially easy to grasp the situation by comparison with the corresponding chromosome in the row above, where, if we imagine the free ends brought into contact, the two conditions would be very similar.

At first thought it may seem to some readers that the long free arms may be due to a shift of fiber attachment. Such a condition is carried in one of the families to be taken up later; e.g., the male used in mating number 14 (pl. 3, row 15) carries one homologue of this seventh pair which has a third position for fiber insertion so that when opening out in the usual way this free end is about twice as long as the type form. Furthermore, this pair also opens out either way, giving figures such as that

seen in text figure A where the longer arms of both homologues are free. This is one of the clearest instances with which I am familiar where either the long or short ends of a given atelomitic tetrad become free. Wenrich ('17, p. 488) has noted and discussed variation in method of transformation of certain tetrads in another species. I can only agree with his conclusion that chromatid movements cease at a certain stage of the prophase and are not resumed until the metaphase. The movements of the chromatids during the early prophase have considerable range of variation, consequently any particular tetrad may enter the metaphase in a number of forms as will be evident to any one who cares to examine critically the four largest tetrads in the appended plates. While any two of these chromosomes might



Text fig. A Drawings of tetrad no. 7, showing the various forms in which it has been found. Four individuals represented. 1 and 2, the typical short-armed form which occasionally opens in the same specimen with the long arms free; 3 and 4, shows the same condition in an individual in which there has been a secondary shift of point of fiber attachment on one homologue; 5, very rare telomitic form; 6, heteromorphic form.

be interchanged in their position in the size series, none of these four would be confused with the other chromosomes of the complex, except with number 8 in the two individuals where it is atelomitic (rows 18 and 24). In the complexes represented in rows 12 and 17 all four are axial rings which are very similar in appearance, whereas the same four chromosomes occur in a variety of forms throughout the plates.

We will consider only one modification—the E-shaped tetrads. Those familiar with first spermatocyte prophase conditions know that the larger chromosomes usually form double or triple rings at this stage, each successive loop being at a right angle to the preceding one (Sutton, '02). Such tetrads frequently enter the metaphase in corresponding forms with rings at angles to each

other like those represented in column 12, rows 7, 24, 26, and 28. There seems, however, to be a tendency for the small ring of such figures of 8 to rotate  $180^\circ$  so that it comes to lie within the larger ring which at the same time rotates resulting in a form such as that represented by chromosome 10, row 15. Obviously, the ends of the smaller inner ring would separate early in the ensuing division resulting in E forms like those represented in column 12, rows 4 and 21. Those interested will find other transition forms shown in the plates.

*c. An octad multiple.* When the first two species of *Circotettix* studied showed twenty-one chromosomes in the spermatogonia and eleven in the first spermatocytes, it was at once surmised that one of the four largest first spermatocyte chromosomes is an octad. This assumption has been verified by the breaking down of the octad into its component tetrads in a few cells of one of the male offspring of mating number 5. Rows 13 and 14 represent two complexes from this individual. In the latter of these two cells there are twelve chromosomes, eleven tetrads and the accessory. There are three instead of the usual four large atelomitics. The place of the missing atelomitic (column 11) is occupied by a telomitic tetrad which would come about seventh in the size series as arranged, while the other member of this potential octad combination appears as the lacking number 3.

It was the conviction that one of the small pairs of chromosomes, either number 2 or number 3, had entered into a multiple formation with one of the pairs of intermediate size rather than that this chromatin had been lost to the complex in the genus *Circotettix* which caused me to leave ('17) a blank column in the plate showing a serial arrangement of the chromosomes of another species of this genus.

Row 13 represents another complex from the same individual. The octad here is only partially broken down at one end (column 11). A similar condition is shown in row 18, column 11. The resemblance of this figure to the octad multiples of *Hesperotettix viridis* is striking, as may be seen by comparison with the photomicrographs by McClung ('17), plate 8, figures Q to T.



*Circotettix verruculatus*, then, does not constitute an exception, so far as number of chromosomes is concerned (when the total number of chromatids in the complex is taken into consideration), to the typical conditions in the Acrididae. And since the complex is essentially similar in the several species of *Circotettix*, so far studied, it seems safe to extend this conclusion to the genus.

*d. Differential frequency of the atelomitic condition of the three critical pairs.* In order to determine whether the offspring of a particular mating were giving the range of chromosomal variation of the species, or that to be expected from a union of the gametes of their parents, it was necessary to find approximately the normal frequency of any given condition for each member of the three pairs numbered 1, 7, and 8.

A study of forty wild males showed that tetrad number 1 was telomitic in thirty-two individuals, atelomitic in two, and heteromorphic in six—a ratio of 70 telomitic to 10 atelomitic homologues. Tetrad number 8 paralleled this condition closely, since in thirty-three animals it was telomitic, in one atelomitic, and in six heteromorphic, giving the ratio 72 telomitic to 8 atelomitic dyads. Tetrad number 7, on the other hand, practically reversed conditions, since in thirty-one animals it was atelomitic, in one telomitic, and in eight heteromorphic—a ratio of 70 atelomitic to 10 telomitic dyads. The situation is summarized in table below.

	TETRADS			DYADS		
	Telomitic	Heteromorphic	Atelomitic	Telomitic	Atelomitic	Ratio
1	32	6	2	70	10	7:1
7	1	8	31	10	70	1:7
8	33	6	1	72	8	9:1
	66	20	34	152	88	1.7:1

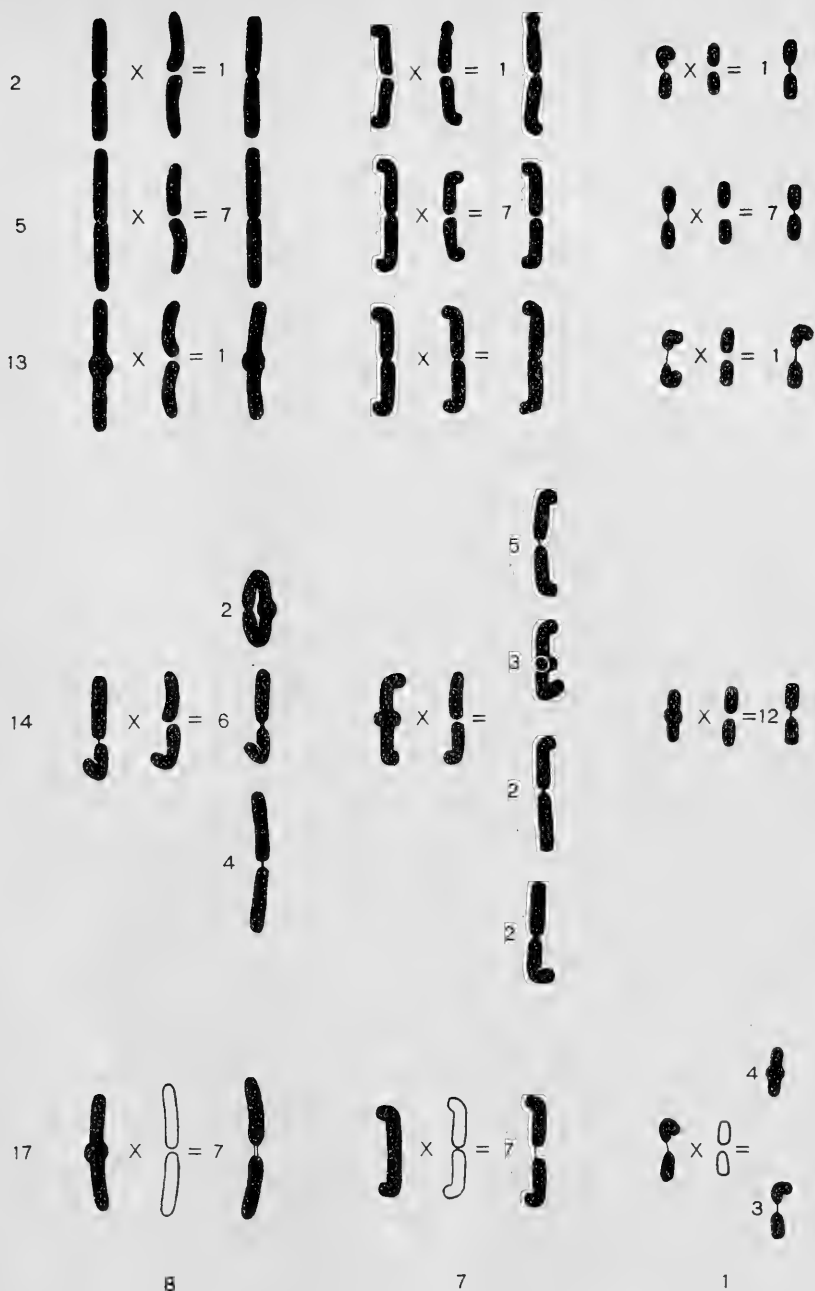
Comparison of the ratio of the sum of the telomitic to the atelomitic dyads of the three pairs which is roughly two to one with that of the individual pairs shows clearly the necessity of dealing with each pair separately.

*2. Description of chromosomal complexes involved in specific matings*

An analysis of the male progeny only has been made because the evidence which they furnish is believed to be sufficient and it is free from any uncertainty in identifying homologues, since the figures represent first spermatocytes with the pairs synapsed. Two matings, each with a single male offspring, are shown on plate 1 because both of these matings involved the rather rare condition of atelomitic homologues in pair number 1. Text figure B gives the form of each of the three pairs of chromosomes under consideration in both parents and progeny of all of the matings.

*a. Mating number 2 (pl. 1, rows 1 and 2 ♂ parent, row 3 ♀ parent, row 4 ♂ offspring).* The chromosomal complexes involved in this mating are shown in horizontal rows 1 to 4. The first two rows represent, respectively, first spermatocyte and spermatogonial complexes. The latter is given chiefly to enable the reader to make a more direct comparison with the diploid complex of the female (row 3). The most striking difference between these two complexes appears in column 6, the presence of two accessories in the female complex and one in the male. Row 4 represents a first spermatocyte complex of one of their offspring.

Taking up the three critical pairs numbers 1, 7, 8, we see: 1) that number 1 is heteromorphic in the male and telomitic in the female. If, then, the point of fiber attachment remains constant from parent to offspring, the chances are equal for this chromosome to be either heteromorphic or telomitic in the progeny, while an atelomitic pair would be impossible. In the one male offspring from this cross both homologues were telomitic. 2) Chromosome number 7 was atelomitic in the male and heteromorphic in the female. The chances were, therefore, one to one of its being either atelomitic or heteromorphic in a given offspring. In the one male obtained it was heteromorphic. A further point which may be mentioned here is that the telomitic homologue, which on the assumption above mentioned is



Text fig. B. A condensed arrangement showing the morphology of the three critical pairs of chromosomes in the parents of each of the five matings and in their male offspring with the number of individuals in each class. The chromosomes of the male are presented on the left; those of the female on the right. In mating no. 17, where the form of the chromosomes of the female can only be hypothesized, they are represented in outline.

derived from the mother, shows a constriction near the end to which the fiber attaches in both parent and offspring. This is a condition which I have noted before ('17) as running through the complexes of given individuals. The only objection to the use of this characteristic as a tag is that it is not always apparent in individuals where it potentially exists. 3) Chromosome number 8 was telomitic in both parents and in the offspring.

*b. Mating number 13 (pl. 1, row 5 ♂ parent, row 6 ♀ parent, row 7 ♂ offspring).* The chief interest in this mating as already stated lies in chromosome number 1. In the father both homologues are atelomitic, in the mother telomitic, so that the offspring would be expected to be heteromorphic, if there is no reorganization. That such is the case may be seen in row 7. Chromosome number 7 is atelomitic in both parents and offspring and chromosome number 8 is telomitic in all three.

*c. Mating number 5 (pl. 2, row 8 ♂ parent, row 9 ♀ parent, rows 10-14 ♂ progeny).* Seven male offspring were studied. All had similar chromosomal constitutions. First spermatocyte complexes of five of these are shown on the plate. Both parents have the three pairs of chromosomes under consideration in the condition in which they most frequently recur in the species, but if the chromosomes of their progeny vary according to their range in the species the other conditions might appear, e.g., chromosome number 1, which is telomitic in the parents, ought, if its telomitic and atelomitic phases follow their frequency of occurrence in the species, to have given two atelomitic dyads among the fourteen contained in these seven individuals, since we have seen that the ratio among the wild individuals is 1 to 7. Likewise, one out of every nine homologues of pair number 8 should have been atelomitic and one out of seven dyads of pair number 7 should have been telomitic. In no cases was there a change from the type of chromosome transmitted by the parents. To be sure, the number of individuals is small, but when we consider that the expectations would have been about two variations for each of the three pairs or six chances the evidence decidedly favors the alternative hypothesis of constancy of form. (As has been mentioned before, chromosome 7, row

12, has opened with the long instead of the short arms free, but this is a different type of phenomenon.)

*d. Mating number 14 (pls. 3 and 4, row 15 ♂ parent, row 16 ♀ parent, rows 17-28 ♂ offspring).* This is the most interesting mating obtained so far, on account of one homologue of the seventh pair in the father having a nearly median fiber attachment, so that one free arm is about twice as long as the other when the tetrad opens in the more usual manner. This condition is constant in the male parent. The other homologue of this pair has the usual short free arm, so that the combination is heteromorphic and atelomitic. In the mother the corresponding pair is also heteromorphic; one member being telomitic, the other atelomitic, so that four combinations are possible in the offspring (text fig. B). Theoretically, these should occur in equal numbers; for the twelve male offspring we should expect a 3:3 : 3:3 ratio, actually it was 2:3 : 5:2, but when we take into account that these are the recombinations in the tetrads of such a small number of individuals, the result is as close as can reasonably be expected. A more direct comparison is between the number of homologues of each of the three types expected and the number actually obtained. Since the father contributes one long-armed atelomitic and the mother one telomitic, while both parents contribute a short-armed atelomitic, we should expect a ratio of 6:6:12. The actual ratio is 5:4:15.

Chromosome pair number 8 is also heteromorphic in both parents. One would therefore expect twelve of each of the two types of homologues. The numbers obtained are ten atelomitic to fourteen telomitic. The combinations in the tetrads where one would expect 3:6:3 are 2:6:4.

Pair number 1 is telomitic in both parents and in all of the offspring studied. Had the two types of homologues of this pair followed their respective frequency of occurrence in the species, four homologues should have shifted to the atelomitic condition.

*e. Mating number 17 (pl. 5, row 29 ♂ parent, rows 30-35 ♂ offspring).* The female used in this cross died, hence knowledge of her complex was not obtained, but if we accept the foregoing evidence that the architecture of the chromosomes is transmitted

unaltered from parent to offspring it is easily seen by comparing the complexes of the seven sons with each other and with that of the father that pair number 7 was atelomitic in the mother, pair number 8 was telomitic and pair number 1, which is heteromorphic in the father and also in three of the sons and telomitic in four, must have been telomitic in the mother.

#### DISCUSSION

Since the rediscovery of Mendel's laws, cytologists have accumulated a mass of evidence demonstrating the parallelism between the behavior of the chromosomes and the physical mechanism necessary for carrying into effect the known laws of heredity. The existence of the chromosomes in a duplex size series, the union of the members of a pair at synapsis, and their separation during one of the maturation divisions are about as clear evidence as could be expected.

The writer in 1913 was the first to report the occurrence of homologous chromosomes within a species which could be identified one from the other, owing to a size difference, and to show that the members of this pair segregate freely in relation to the accessory. Wenrich ('14), Vořnov ('14 a), and Robertson ('15) reported similar conditions in other Orthoptera. All of these works, however, dealt with the distribution of a single pair of homologues in regard to sex, since the accessory in these forms marks the male-producing from the female-producing spermatozoon by passing undivided to one pole at the first maturation division. In my work on *Trimerotropis*, summarized at the beginning of this paper, the same principle of random segregation was shown to apply to three of the eleven euchromosome pairs. Mr. Robert L. King, working in our laboratory on a still more favorable species, has been able to extend these observations considerably further.

Since the form of a given homologue is constant for the individual, the segregation of the homologues of a particular tetrad and the independent assortment of homologues from several different tetrads was clearly established by the intensive study of chosen individuals. The points which remained to be deter-

mined were: whether or not the morphological constitution of the chromosomes is transmitted from parent to offspring, and, if so, whether their recombinations in the progeny are according to the laws of chance.

Among these twenty-eight male offspring of five crosses there are fifty-six homologues ( $2 \times 28$ ) for each of the three pairs of chromosomes (nos. 1, 7, 8) or a total of one hundred and sixty-eight homologues ( $3 \times 56$ ) in all, which might have varied at the ratio found in the wild individuals of one atelomitic to seven telomitics for pair 1; one atelomitic to nine telomitics for pair 8, and of one telomitic to seven atelomitics in pair 7. One would expect twenty-two shifts of fiber attachment in such a number of homologues if there is a reorganization during ontogeny which repeats the conditions in the species. On the contrary, not a single offspring varied in its chromosomal constitution beyond the limits to be expected from a combination of the gametes of its parents. In other words, any given chromosome reappeared in the progeny in the same form that it possessed when it went into the parental gamete. This is shown more clearly by comparing families which carry atelomitic number 1's with those where the number 1's are telomitic. In the former (matings 2, 13, 17, text fig. B) there are fourteen telomitics to four atelomitics (ratio 7 to 2) in the latter (matings 5 and 14, text fig. 2) there are thirty-eight telomitics to no atelomitics.

The same thing is shown by pair number 8; in four families (numbers 2, 13, 5, 17) involving sixteen offspring, the thirty-two homologues are all telomitic; whereas among the twenty-four homologues of this pair in family number 14, where both parents are heteromorphic in this respect, ten are atelomitic and fourteen telomitic.

These data I believe are sufficient to establish the constancy of point of fiber attachment from parent to offspring for this group, and to lay the basis for further work on the assumption that structural variations in the chromosomes are correlated with somatic characters in such way that it will be possible to tell what the chromosomal constitution of a wild individual is in regard to a given pair from an external study of the animal.

At all events, in *Circotettix verruculatus*, we can say in regard to the chromosomes which enter the gametes, just as certainly as of a pair of contrasting unit characters which segregate in the  $F_2$  generation, that *this* one was contributed by the father, *that* one by the mother.

In genetical work, unless there is sex-linkage or, as in the case of Nabour's *Paratettix*, ('14, '17) incomplete dominance, the  $F_2$  generation must be obtained before an analysis of the gametes of the grandparents can be made. In this work a cytological analysis is made of the chromosomal complexes of both parents, so that we have definite knowledge of what goes into the  $F_1$  generation. Since there is no reorganization, an analysis of the chromosomal complexes of the  $F_1$  generation gives us at once what in genetical studies becomes evident only on a study of the progeny produced by the union of these gametes. In other words, the chromosomal characters dealt with are naturally studied in the germ cells, and it is obvious that the gametes of the  $F_1$  generation would, on fertilization, become the  $F_2$  zygotes. So that, while one would have to wait for the  $F_2$  individuals to analyze somatic characters, the corresponding analysis of the chromosomes is made on the maturing gametes.

Thus, with an actual identification of the homologues of given pairs of chromosomes from parents to offspring and with a determination of their ratios of recombination, the parallelism between the behavior of the chromosomes and Mendelian phenomena is complete.<sup>4</sup> So able a student of heredity and environment as

<sup>4</sup> Judging from a recent paper by Dr. Harmon ('20), based on Dr. Nabour's pedigreed *Paratettix*, this form may be favorable for a similar line of investigation. She reports that the type BB has the ends of the third pair of chromosomes hook-shaped and the type CC has this pair rod-shaped, while BC, the hybrid, has one chromosome of each sort.

There are two obvious weaknesses in her report. There is no statement as to whether father and sons were examined or whether examples of the general population were taken. Nor are there any data as to the number of individuals of the various types studied.

In any event, it is not shown that there is a correlation between the color pattern and the morphology of this chromosome pair; such a relationship would be suggested if in the  $F_2$  BB and hook-shaped number 3's and CC and rod-shaped number 3's segregated together.



E. G. Conklin ('20) closes a recent article on the mechanism of evolution with the following sentence: "We may therefore conclude that the Mendelian law of heredity, especially as regards segregation of inheritance factors is of universal occurrence—that there is no other type of inheritance."

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## EXPLANATION OF PLATES

The complexes were drawn with the aid of a camera lucida at a magnification of 2400 diameters. In the reproductions they appear at 1600 diameters. The chromosomes were rearranged for the plates roughly according to size by tracing from the original drawings.

The haploid complexes are from side views; the diploid complexes from polar views. Each horizontal row represents the chromosomes of one cell, each vertical row corresponding chromosomes from different cells. The arrangement is such that the accessory, no. 6, is always passing to the upper pole.

Column 3 is left vacant since the member which belongs here is united with one of the others to form an octad.

### PLATE 1

#### EXPLANATION OF FIGURES

1 to 4 Complexes involved in mating no. 2.

1 First spermatocyte complex of father; pair no. 6 atelomitic, pair no. 1 heteromorphic.

2 Spermatogonial complex of father.

3 Somatic complex of mother; pair no. 6 heteromorphic, pair no. 1 telomitic.

4 First spermatocyte complex of a son; pair no. 6 heteromorphic, pair no. 1 telomitic.

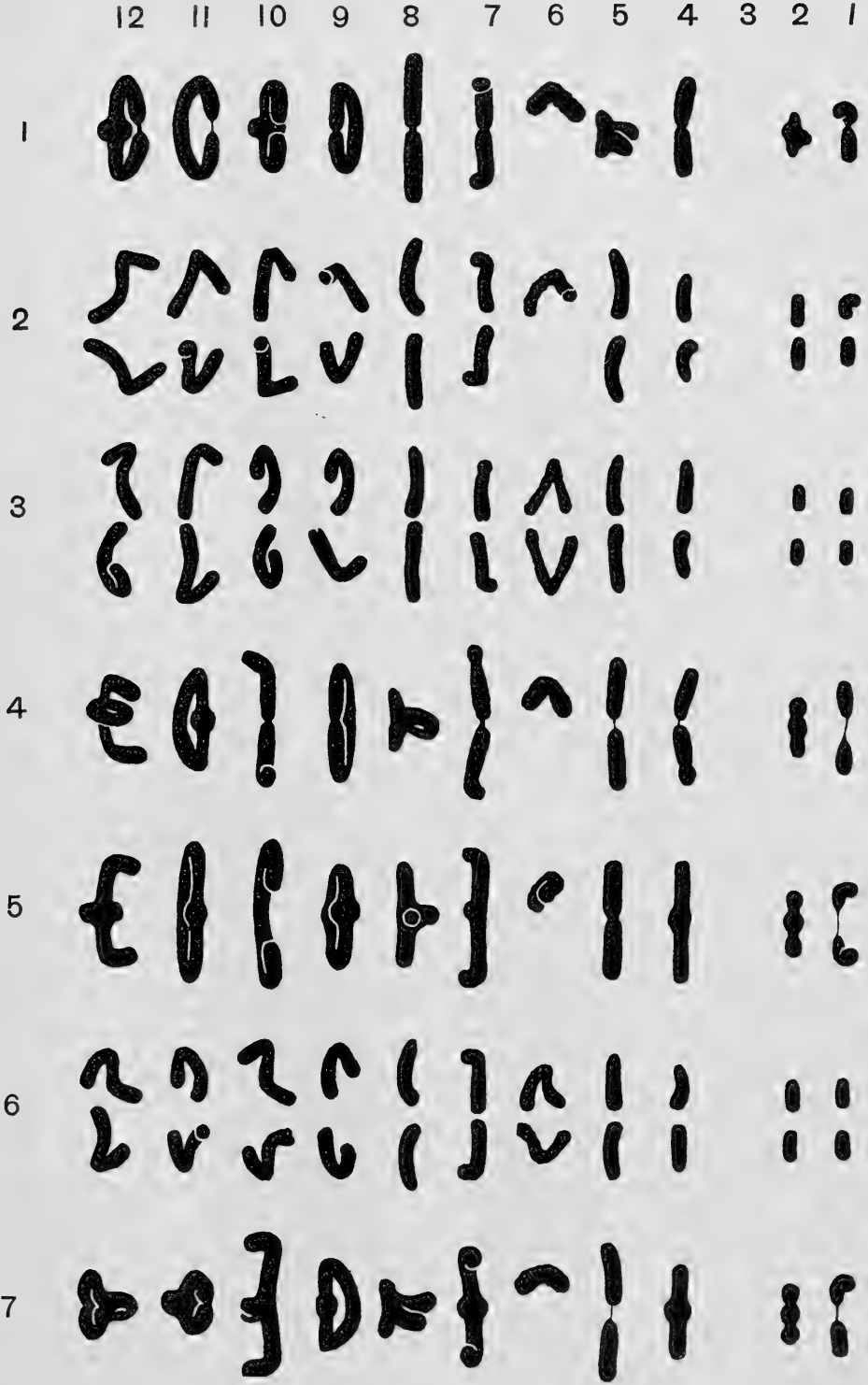
5 to 7 Complexes involved in mating no. 13.

5 First spermatocyte complex of father; pair no. 1 atelomitic.

6 Somatic complex of mother; pair no. 1 telomitic.

7 First spermatocyte complex of a son; pair no. 1 heteromorphic.

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## PLATE 2

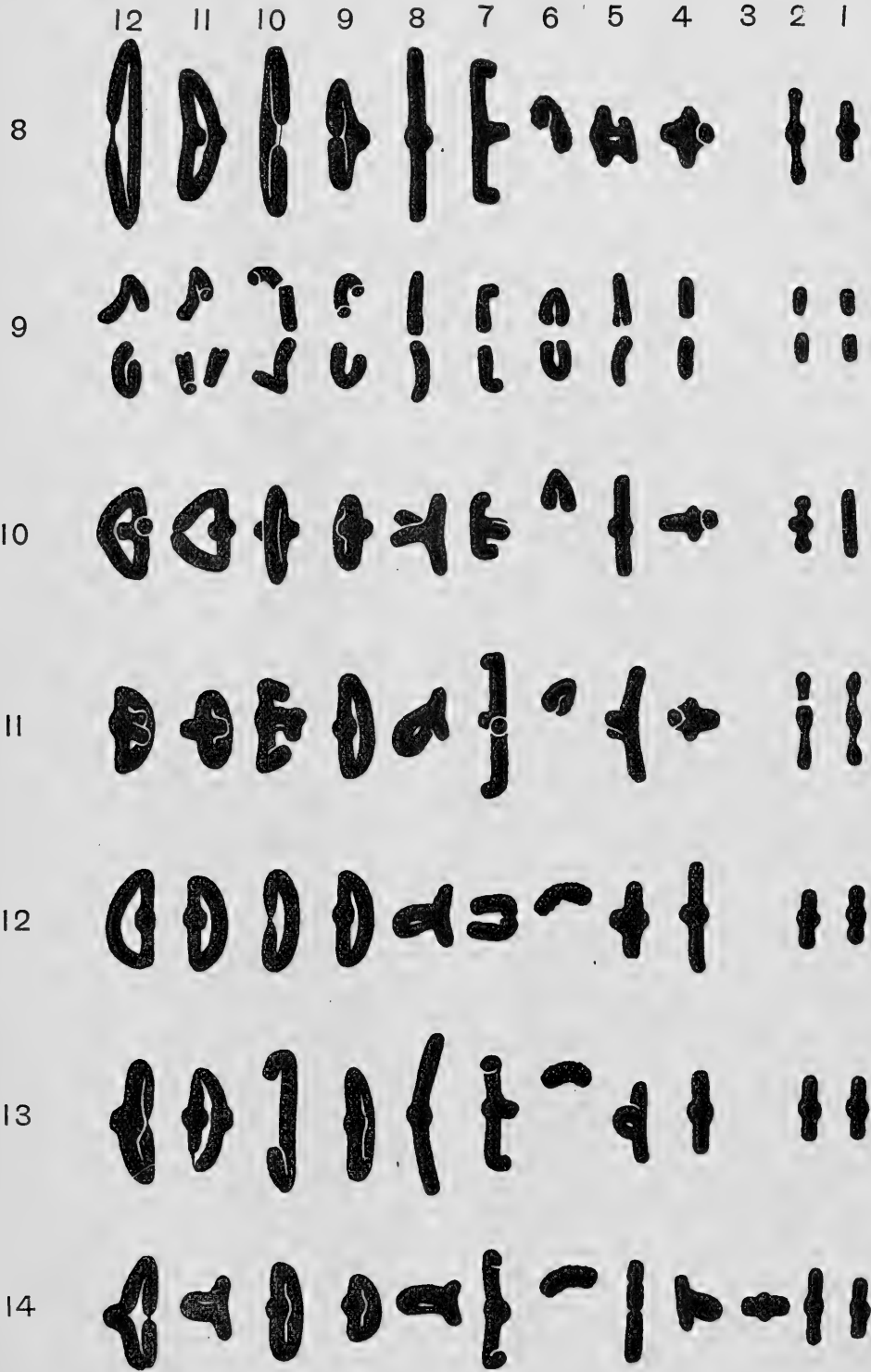
### EXPLANATION OF FIGURES

8 to 14 Complexes involved in mating number 5.

8 First spermatocyte complex of father.

9 Somatic complex of mother; complex in two sections, two chromosomes cut (columns 10 and 11).

10 to 14 First spermatocyte complexes of four sons, 13 and 14 are complexes from the same individual; in 13 the octad multiple (no. 11) is partially disassociated at one end, in 14 it is completely disassociated, the component tetrads being shown as no. 3 and no. 11. Note that the three tetrads nos. 1, 7 and 8 are of similar form in both parents and that there is no variation from the parental forms of these chromosomes in the offspring. Tetrad no. 7, row 12, had opened with the long instead of the short arms free.



## PLATES 3 AND 4

### EXPLANATION OF FIGURES

- 15 to 28 Complexes involved in mating no. 14.
- 15 First spermatocyte of father; tetrad no. 7 is a heteromorphic atelomitic.
- 16 Somatic complex of mother; pairs no. 7 and no. 8 heteromorphic.
- 17 to 28 First spermatocyte complexes of twelve sons; tetrads no. 7 and no. 8 show the various combinations in different individuals to be expected from a free union of the gametes of the parents.

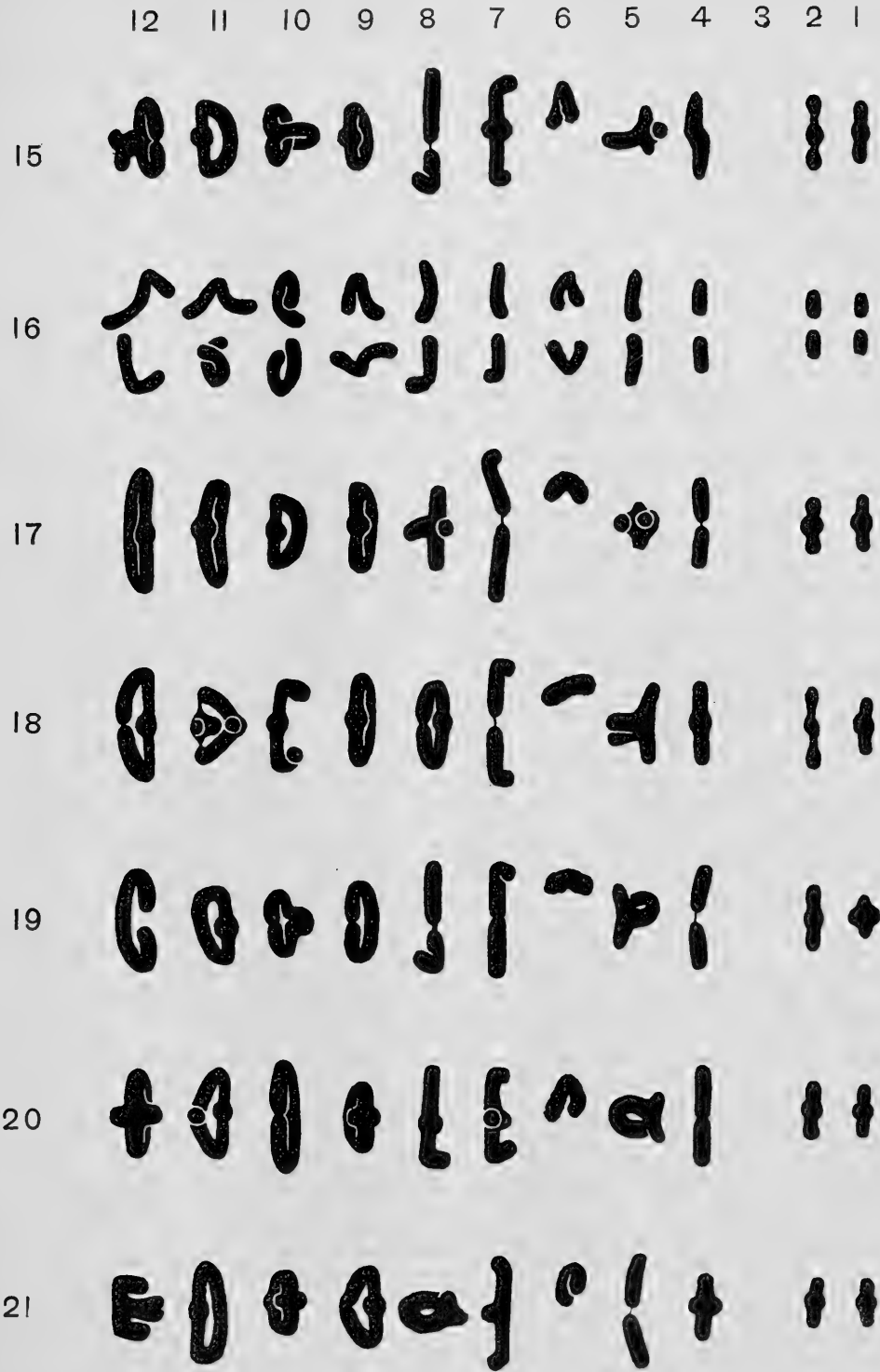
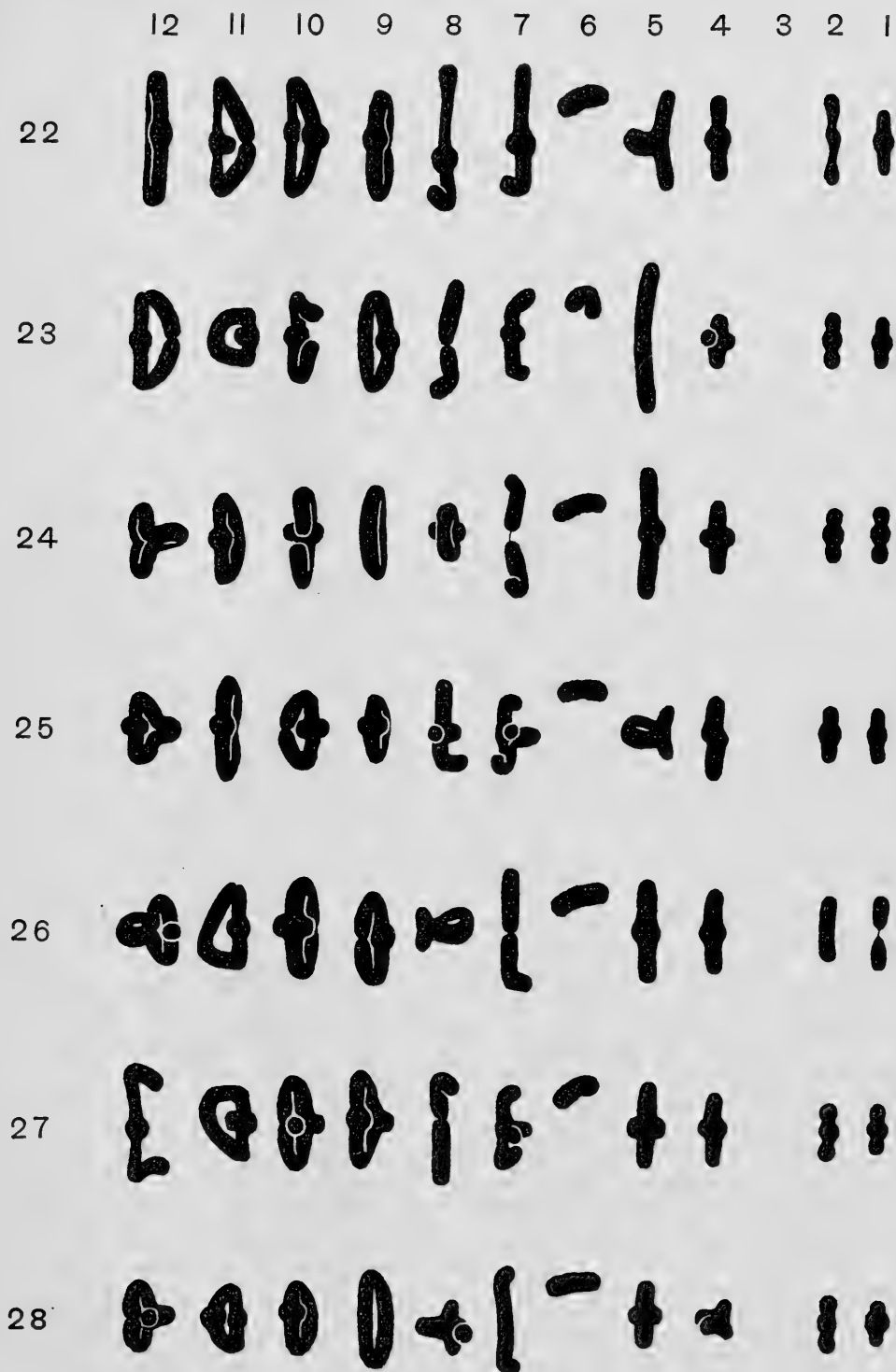


PLATE 4

See description of preceding plate



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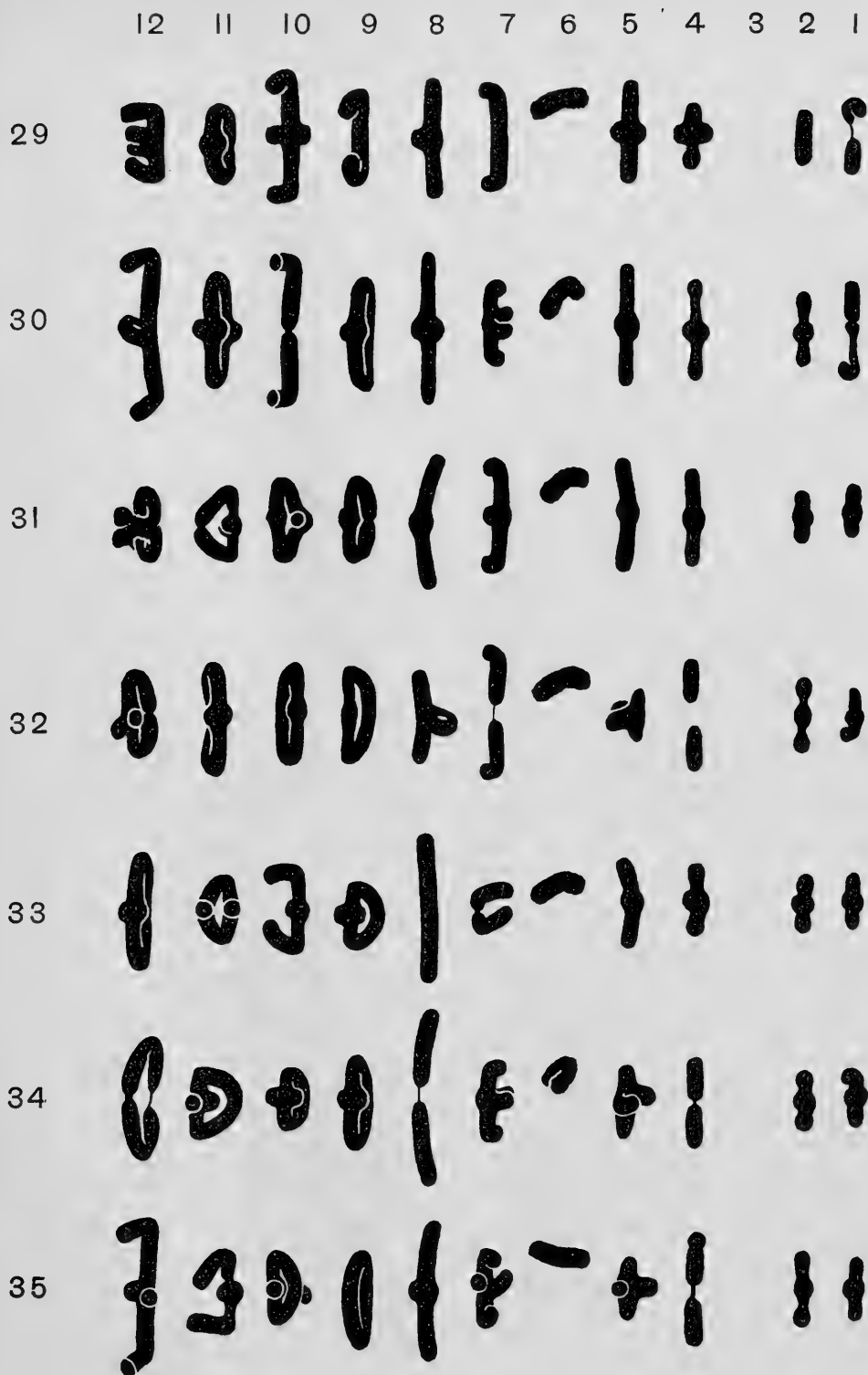
## PLATE 5

### EXPLANATION OF FIGURES

29 to 35 Complexes involved in mating no. 17.

29 First spermatocyte complex of father.

30 to 35 First spermatocyte complexes of five sons; pair no. 1 must have been telomitic in the mother to give these results.





# CYTOLOGICAL STUDIES ON THE INTERNAL SECRETORY FUNCTIONS IN THE HUMAN PLACENTA AND DECIDUA

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TWO DOUBLE PLATES (NINETY-SIX FIGURES) AND ONE TEXT FIGURE

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## INTRODUCTION

In recent times, along with an increase of knowledge on internal secretion, it has been imagined by many authors that in the placenta and the decidua there should exist such a function. According to the literature which I know, Lettule and Larrier ('01) are those whose attention was first drawn to this subject. They detected in the syncytium layer a kind of granular body termed 'Plasmoidale Kugeln.' This body they took to be a secretion of the placenta. Subsequently, Veit ('02) attempted to trace the cause of eclampsia, and Behm ('03) the cause of morning-sickness to the function of the syncytium, and Bouchacourt ('03) observed an increase of lactation by using some placental preparations. Further, Halban, supported by his plentiful clinical observations, stated that the swelling of the nipples noticeable in a newborn child, the congestion and hyperplasy of the uterus, the hypertrophy of the mammary glands of a pregnant mother, and hypertrichosis are all probably due to his so-called 'Reizstoff,' which is thought to be a product of the placenta. Among other things, it seemed he tried to deduce the existence of the closest functionary correlation between the placenta and the mammary glands. Since the result of Halban's studies was published, the functions of internal secretions likely to exist in the placenta drew general attention, and thereafter studies of this subject followed quickly one after the other. In the present essay I have refrained from chronologically relating all the results of these researches, but instead, with a view to giving only a general idea of what is known about this subject at present, I have confined myself to the summing up of all the points of the investigations made by the various authors up to now, and to dividing them into a few large sections along the lines of experimentation, biological chemistry, and histology.

Now, in the early experimental investigations, it was the first attempt of the various authors to investigate chiefly the effect which the placenta has upon the mammary glands, and the methods employed were either to inject into the animal used as the subject of the experiment an extract of the placenta

or to transplant the placenta, and the results in either case were for the most part positive (Feller, '09; Lederer and Pribram, '10; Aschner and Grigoriu, '11; Cristea and Aschner, '12; Basch, '12; G. Kawaida, '12; M. Dohi, '16). However, as it was incidentally discovered at the same time that the placenta had shown some strong special reaction upon the other organs, e.g., the vascular organs (Schickele, '12), the internal secretory glands (Fellner, '13; Colle, '13), and the uterus (Fellner, '09; Okintschitz, '14; Hermann, '15), it naturally became difficult to assert that the ingredients of the placenta have such properties as react upon the mammary glands alone. And, further, as it became known generally that such changes in the mammary glands as mentioned above were not only due to pregnancy or the placental ingredients, but were also caused by extracts of the various organs, such as the embryo (Mandle, '05; Bayliss and Starling, '06; Foa, '10; Biedl and Königstein, '10; Aschner and Grigoriu, '10), the ovary (Ott and Scott, '10; Aschner and Grigoriu, '11; Frank and Unger, '11; Hermann, '15; Y. Taniguchi, '16), the pituitary body (Hofstätter, '11), the pineal and thyroid glands (Ott and Scott, '11), the uterus while in child-bed and the mammary glands which are giving suck (Schoefer and Mackenzie, '11), the intestines, the testicles, the spleen, and the thymus (Kehrer, '15), the decidua (Gentili and Bina-ghi, '17), as well as by a certain kind of chemical matter, such as 'Lymphagoga' (Aschner and Grigoriu), and albumin, too (Frank and Unger, '11; Fraenkel, '14), the theory of internal secretion in the placenta which had begun to be adopted generally for a time began to lose its value by degrees.

In the next place, along lines of biological chemistry, there are the researches made by such authors as Higuchi ('09), Hermann ('15), and Harada ('16). These authors extracted from the placenta various kinds of chemical matter as its principal ingredients and subjected them to close examination, but they never mentioned a 'hormone,' which is always existent in the placenta.

Thirdly, with regard to the histological studies of the placenta, many researches have been made in the subject from earlier

times, but a great majority of these researches were confined to the development or general construction of the placenta, and very few authors have given any special consideration to the internal secretion of this organ nor have they closely examined the minute construction of the tissue elements or cells of the placenta. Already Ahlfeld ('78) had noted the appearance of vacuoles in the syncytium layer, and subsequently many authors recognized it; however, regarding the thing itself and its physiological significance, nothing definite has yet been stated. Gottschalk ('90) deemed it a pathological product; Kossman ('92) took it for a kind of degeneration, while Langhans ('92) interpreted it as 'Leichenerscheinung.' Further, regarding the globules of fat in the same layer, demonstrations had already been made by Pela-Leusden ('97) and Marschand ('98). Bonnet ('99) deemed it a nutritious matter taken up by the embryo, and in recent times authors have generally agreed in recognizing it as nutritious matter of the embryo taken from the mother's blood. Others, among them Halfbauer ('05), Costa ('04 and '05), and Bondi ('11), inferred that the appearance of this matter must be due to the active functions of the cells, by which it is assimilated and absorbed. On the other hand, however, Wolff ('13), as per Letulle and Larrier as mentioned above, on comparing the granular body of the syncytium layer with the secretory grains within the ordinary gland-cells, stated that both of them were a similar production. This theory was subsequently supported by Fraenkel, who was close to the deduction that there might be existent a certain secretory relation in the granular body of the syncytium layer. In short, the histological studies of this subject up to now have been confined, as mentioned above, to the construction and, consequently, the functions of the syncytium layer only, and there are even many defects in the studies and some resultant weakness in the point of the arguments, and no one can say that the opinions agree. Although there are some authors who recognized the internal secretory functions of the placenta, yet, since their arguments are based on a single part of the organ, viz., the syncytium layer, it must be stated that on the whole the basis of histological



arguments for internal secretion in the placenta is very feeble indeed, and much more is still to be done to accomplish a consummate investigation of this subject. It should none the less be added that Fraenkel ('14) was one of those who were strongly opposed to the various theories given above, and he denied the effects, which, it was believed, the placental functions have upon the mammary glands, on the ground that exactly the same phenomenon as takes place in the suckling of mammals was observed in the suckling of Monotremata and Marsupialia, neither of which has the placental formation. In addition to that, Fraenkel, going so far as to discuss the changes which the internal secretory glands and other organs have to undergo in consequence of the placental extract as was witnessed in the experimentations of the afore-mentioned authors, concluded that such changes were attributable to several ingredients, his so-called 'Gift,' contained in the extract. According to Fraenkel, who contradicted the theory which generally is in favor of the existence of internal secretion in the placenta, the internal secretory glands first respond to this 'Gift,' and, as a result, the other physical changes follow. In short, it may be said with regard to the existence of internal secretion in the placenta that, in spite of the numerous researches made along the lines of experimentation, biological chemistry, and histology, as I have mentioned above, the views expressed are so varied that no conclusion has been reached, and, therefore, regarding the real situation of this subject, it is at present not yet an established theory.

Many authors have given their attention to the existence of an internal secretory function in the decidua, and have tried to demonstrate it, Starling ('06), Sfameni, Gentili ('13 and '14), Schöttländer ('14), Aschheim ('15), Gentili and Binaghi ('17) being among the number. Starling conducted experiments on the rabbit to find the effects which the juice extracted from the mucous membrane of the uterus while in pregnancy exerts upon the mammary glands, and the result was of a negative nature. Sfameni for many years past had held that in the decidual cells there should be existent an internal secretory

function, the basis of his arguments being the striking resemblance from the view-point of morphology, between the decidual cells and those of the other internal secretory glands. In order to demonstrate this, Gentili, in the same laboratory as Sfameni, carried out experiments by the use of the decidual juice on the dog, the rabbit, and the frog, and it was found that, similar to the luteal cells of the ovary, the decidual cells have a special action upon both the circulatory and generative organs. Schöttländer also, by assuming that it may be possible for the secretion of the glandular cells in the spongy layer of the decidua in the first month of pregnancy perhaps to enter the mother's blood directly, recognized seemingly that the uterine glands at times have the significance of an internal secretory gland. Aschheim, in 1915, by discovering plenty of lipid in the decidual cells, imagined the existence of a special function in these cells. Lastly, Gentili and Binaghi, by conducting a microchemical experiment on the various tissue elements which form the decidua of a cow, demonstrated the existence of a kind of lipid in the tissues, as reflexive of the correlation which it seems they believed existing between a certain function, particularly an internal secretion of the decidual cells, and the lipid. On a close examination of the result of researches made by these authors, it must be stated that even in those whose view was in favor of an internal secretion in the decidua the basis of arguments presented is in general as feeble as in the case of an internal secretion of the placenta.

It is a long time since I began to feel interested in the secretory function of the placenta and the decidua. I was ever of the opinion that both the placenta and the decidua have a certain secretory function, and the experimental method I adopted in investigating this subject was entirely different from that employed by the foregoing authors. That is to say, I conducted a serological research into the effects which the alcoholic extract of the placenta and the decidua has upon the mother's blood, and the result was the discovery in the serum during pregnancy of, not only the well-known Abderhalden's 'Abbauf ferment,' but also of such an antibody as has a property of the fixation of complement with ingredients of the extract

referred to. And, as in ordinary cases, the antigen which represents the phenomenon of fixation of complement is either a proteid or some such like matter, whereas in my case it is a substance which is soluble in alcohol, the latter must be deemed to be qualitatively different from the former. It makes me feel as if it were in order for me to presume that the antigens in my case are due to the lipoid substances which are peculiar to (having some relation with the secretory process of) both the placenta and the decidua. Accordingly I have become convinced that, in spite of the various authors whose arguments I have enumerated above as denying the existence of an internal secretory function in both the organs concerned, the fact must be the reverse. Needless to say, however, this argument is only along lines of reasoning, and, therefore, it must be stated that the reason why I have applied at this juncture the modern cytological methods, thus planning out a close histological investigation of the principal tissues and cells, particularly the cell bodies of the placenta and the decidua, was because I was anxious to decide more clearly the right or wrong of this hypothesis. I have also, by adopting the same methods, examined the minute structure of the principal cells as indicative of the change which the uterine mucous membrane undergoes prior to menstruation, which, being compared with that during pregnancy, has enabled me to arrive at a certain conclusion, as will be noted later, respecting the physiological significance of the menstrual changes of the uterine mucous membrane.

#### MATERIALS AND METHODS

The materials for research were taken from a total of forty-three cases, of which twenty-five had to undergo artificial interruption during the first half of pregnancy because of the following diseases: 6 cases of morning-sickness of high degree, 12 cases of phthisis, 2 cases of laryngeal tuberculosis, 1 case each of consumption of the bowels, of peritoneal tuberculosis, and of a valvular disease of the heart, 2 cases of glucosuria.

Of the remaining 18 cases, 14 had to undergo artificial interruption during the second half of pregnancy because of the follow-

ing diseases: 1 case of beriberi, 5 cases of nephritis, 2 cases of a valvular disease of the heart, 1 case of albuminuric retinitis, 3 cases of eclampsia, 2 cases of placenta praevia.

And the remaining 4 cases had to receive a Porro's operation because of the following diseases: 1 case of mislaid transverse position and 3 cases of the narrow pelvis.

And as to the time of pregnancy in these 43 cases, it should be noted that 4 cases were less than 1 month, 2 cases 1 month, 4 cases 2 months, 7 cases 3 months, 6 cases 4 months, 3 cases 5 months, 4 cases 6 months, 3 cases 7 months, 3 cases 8 months, 4 cases 9 months, and 3 cases 10 months. And difficult as it was to accurately determine the exact time of pregnancy in each case, the method I adopted in determining those less than one month was to take into account the size of the egg and the degree of development of the embryo and villi, and in those one month and upward, to consider the time of menstruation, the size of the uterus, the length and weight of the embryo, the length of the navel string, and the weight of the placenta, thus arriving at the approximate time of pregnancy.

As regards the obtaining of the materials from the fetal placenta, it should be noted that in the early stage of pregnancy a few pieces of ordinary villi from the surface of the ovum were taken, while in a little more advanced a few small cuts of the chorion frondosum, and in a well-formed stage of the placenta a few small bits of the latter have been cut out. Now, as to the maternal part of the placenta, a part was taken from placenta already delivered and from that not grown up yet, in the former case, from the surface opposite the uterus of the placenta, and in the latter, the mucous membrane of the uterus concerned being scratched off quickly, as during an operation it is quite easy for the operator to determine the insertion of the placenta. The tissues obtained in this way have always shown microscopically, besides the proper decidual tissue, the existence in them of parts of villi, syncytium cells, and the deposit of fibrinoid material ('kanalisiertes Fibrin' of Langhans), all of which were proof that they were the materials I desired. In this connection it must be added that the decidual materials

were always taken from the decidua vera only, at every stage of pregnancy, by means of scratching out. All these pieces of tissues were so taken that they should not exceed a cube 3 mm. in size, and immediately after cutting they were dipped in the newly prepared fixing solutions. For the latter I have used: 1, Altmann's fluid of potassium bichromate and osmium tetroxide; 2, Flemming's solution modified by Benda; 3, Flemming's solution modified by Meves; 4, Levi's mixture of formol, osmic acid, potassium bichromate, and corrosive sublimate; 5, Luna's mixture of formol, potassium bichromate, and glacial acetic acid. Each of the pieces was then treated in the usual manner being imbedded in paraffin and cut in sections of 3 to 4  $\mu$ . And as a treatment prior to staining, Rubaschkin's method has been applied in a great majority of cases. For staining I have employed: 1, Altmann's method of acid fuchsin and picric acid; 2, Heidenhain's iron-alum-haematoxylin, and, 3, Benda's alizarin-crystal-violet method, etc. All the methods which I have adopted, the fixation with Levi's mixture, and the staining with iron-alum-haematoxylin have been found to be comparatively successful.

#### MY OWN OBSERVATIONS

The chorionic villi, which are a main component of the placenta, consist of: 1) the syncytium cell-layer; 2) the Langhans' cells, and, 3) the 'Stromazellen,' being cells in the stroma of villi, while the components of the decidua serotina and the decidua vera chiefly consist of, 4) the decidual cells and, 5) the epithelium of the uterine gland. In the present chapter I intend to give a detailed account, mainly from the histological standpoint, of these important tissue components, or cell groups, especially as they appear in the different stages of pregnancy. However, since the structure of all kinds of cells varies even in the same stage of pregnancy and in the same group of cells, it would be a difficult task indeed to explain clearly the correlations existing between the minute structural changes of these cells, or cellular groups, and their functional significance. Therefore, I have

selected and drawn those deemed the most representative of all the structural images of the afore-mentioned tissue-cells which have been widely and thoroughly examined, as will be seen from the series of figures on the plates the object being to give a general idea of the structural changes of these cells. The explanations of each of these figures will make the pith of this chapter, as by so doing I believe the description could be much simplified and its understanding facilitated as much as possible. Thus, I shall appreciate very much if the reader will constantly refer to those figures while reading.

(1). *The syncytium cell-layer (figs. 1 to 12)*

As is well known, the syncytium layer is the epithelium which covers the surface of the villi, and the lack of boundaries between the cells is a feature of this layer. And it is also well known that within this layer there are several kinds of nuclei, differing in size and form and scattered here and there, besides dark-colored granular bodies and a great number of vacuoles which occasionally appeared in it. Its surface is covered with a brush-like border. This layer is generally well developed in the early stages of pregnancy, but in the second half of pregnancy it becomes thinner and looks much like an endothelium, so that, when examining its minute structure, it will be necessary to do so before the fourth month of pregnancy. Figure 1 shows that part of the anchoring villi which extends deeply into the decidua, while all the other plates show the different parts of the surface of the ordinary villi.

In figure 1 the syncytium presents a homogeneous protoplasmic layer generally dark-colored and contains an extremely large quantity of plastosomes (mitochondria). They are of different shapes, but mostly are rod-shaped or bacteroid and of different lengths, the longer ones being slightly curved. They arrange themselves in groups rather than being equally distributed over the layer, and in some places some of them point in the same direction, while others point in various directions,

thus in general giving them something of a meshy arrangement. The nuclei are clear and have in themselves a more or less conspicuous nuclear network, with one or two nucleoli.

In figure 2 the syncytium layer closely resembles figure 1 in structure, though the plastosomes contained therein differ from one another in their shape, arrangement, and number. These two figures show the simplest structure of the syncytium layer.

In figure 3 the plastosomes are generally faint and quite scattered; in the protoplasm there are some dark-colored granular bodies of different sizes and a small number of vacuoles; the smaller granules are somewhat dark in color and are generally found very near the surface, viz., the brush-like border, whereas the larger ones are light-colored and are found in other parts of the layer. The vacuoles are found close to the Langhans' layer. The nuclei are irregular in shape, and besides the nuclear network there are one or two nucleoli.

In figure 4 the plastosomes are generally found in the deeper layer, i.e., close to the Langhans' layer, and they are comparatively small in number. On the contrary, however, plenty of dark or yellowish dark-colored granules occur conspicuously all over the layer, the deeper colored ones being generally superficial. Undoubtedly, granular bodies of this kind have grown up from the dark-colored granules in a conspicuous manner such as I have shown in figure 3 above, and they are very frequently met with elsewhere in the other parts of the syncytium layer. And, moreover, granular bodies of a similar kind are found, as will be seen in the following statement, not only in the syncytium layer, but also commonly in other cell groups. These granular bodies are, of course, extremely varied in their size, quantity, and color; however, since they have a common affinity to certain chemical and coloring matter, e.g. osmic acid, iron-alum-haematoxylin, and acid fuchsin, etc., I have followed, for the sake of brevity, the precedents of many histologists in including all these granular bodies under the name of 'lipoid' granules. In this figure there is, moreover, only one vacuole close to the brush-like border. The nuclei are less conspicuous in their network, the chromatin forming

itself into a large number of lumps, each of nearly equal size.

In figure 5 there are no plastosomes to be found, but the lipid granules occur in extremely large quantities, and their sizes are nearly the same. They are found more or less in groups and are distributed all over the layer. At the same time the vacuoles make their appearance in a conspicuous manner, sometimes on the surface, sometimes in the innermost part, and sometimes in the middle, and they are about the same size as the lipid granules. As is well known, it is in general very difficult to stain the plastosomes every time, and, therefore, accurately to determine their existence and where they are entirely wanting, as in this figure, it would be a very difficult task indeed. I have paid the closest attention to this, and have always selected for it the most excellent preparations for staining, with a view of doing away with all the possible defects in the technique of staining. From the existence, in a very conspicuous manner, of plastosomes in the neighboring tissues, entirely in contrast to this figure, I was prompted to conclude that it was well nigh necessary for me to assert the absolute lack of plastosomes in this part of the syncytium layer. Further, I may add that, as will be noted below, the same amount of attention has been given all the other cells where there are no plastosomes to be found, and I, on this score, am convinced that my observations concerning them are not erroneous.

In figure 6, the surface is somewhat light-colored and is clear. In it there are found innumerable quantities of vacuoles which are nearly of the same size, besides a small number of lipid granules. The innermost part, however, is of a comparatively dark color, and it likewise contains innumerable quantities of minor lipid granules of about the same size, with very few vacuoles which are generally of small size. Some of the nuclei are oval, while others are irregular in their shape, and there is to be found a great number of chromatin granules which make their appearance in lumps of different sizes; the nuclear networks are usually less conspicuous. There are absolutely no plastosomes to be found.



In figure 7 the syncytium layer is extremely thick, and it is difficult to demonstrate the plastosomes. Lipoid granules of extremely varied sizes are found in large quantities. These granules are irregularly arranged, and they tend more or less to occur in groups; certain lipoid granules make their appearance as contents of vacuoles, in which case the granules always have a clear halo around them, as if they constituted the nucleus of the vacuoles. Such images are frequently met with not only in the syncytium layer, but also in other cell groups and, since they are worth recognizing as very clearly indicating the relations which exist between the lipoid granules and the formation of vacuoles, the reader's special attention is hereby drawn to this point (vide the left-hand side of this figure). The vacuoles are abundant, and their sizes and shapes are quite irregular and, as will be seen in the middle part of this figure, a great number of them are joined together at some places without boundaries being noticeable between them. From the existence of such images I am led to infer that an extraordinarily large vacuole is in general the outcome of minor vacuoles being agglutinated and joined to one another. In this way, it seems that the vacuolés which have grown up into tremendous sizes finally rupture toward the surface, as it will be seen in the present figure that such vacuoles open up and connect directly with the intervillous spaces, clearly supporting the interpretation that I have given above.

In figure 8, it is in general extremely deficient in its characteristic dark-colored protoplasm, but then there are all over the layer numberless vacuoles of a very small size, which grow so close to one another that they have exactly the appearance of a beehive. Of these vacuoles some of the larger ones lie together close to the surface, while others, connected with one another, mutually find their outlets to the surface through comparatively large openings. Because of these openings the surface of the syncytium layer, which is naturally level, becomes very uneven and irregular. The lipoid granules are very few, and no plastosomes are to be found. The nuclei are not only

few, but being shrunken are changed into homogeneous bodies of small size, the nucleoli alone making a prominent appearance (vide the right-hand side of the figure).

In figure 9 the syncytium layer is comparatively thin, and there are comparatively few plastosomes to be found, being scattered here and there, and mostly rod-shaped. The lipid granules are middle-sized and are not many in number, and a few of them stay at the center of the vacuoles as if they were the nuclei of the vacuoles. The vacuoles are pretty large, and they arrange themselves close to the Langhans' cells. The nuclei have distinct borders and masses of chromatin arrange themselves in rows, usually close to the nuclear membrane.

In figure 10 the protoplasm is, as in a majority of cases, generally dark-colored, though not homogeneous altogether and, on a close examination, it is found that it contains a great number of vacuoles, which gives the protoplasm more or less a foamy appearance, though very indistinct as compared with other foamy structures. The plastosomes are mostly rod-shaped and are very distinctly stained. They appear generally in the upper layer, though some are noticeable in the innermost layer. There are no lipid granules to be found and no nuclei of normal conditions are to be met with, but, on the contrary, there are some curious bodies, whose size is somewhat larger than the ordinary nuclei and which are irregular in shape. Some of them are homogeneous and are quite dark-colored, while others being non-homogeneous consist of different parts which are either dark or light or somewhat clear, when stained. We come across such structures very often in other parts of the syncytium layer, but so far I have not been able to find out their original nature.

In figure 11 the protoplasm shows numberless vacuoles as its constituents. The vacuoles are somewhat varied in their size, and with the exception of some which are full and stained, a greater portion of them, particularly those which are found on the surface, are more or less loose and somewhat flattened in shape. Between these vacuoles there are extremely large quantities of plastosomes, which are mostly rod- or granular-

shaped and equally deep-colored as these which are found in figure 10. There are few lipid granules to be found, and they exist for the most part in the superficial layer, scattered here and there. What is especially worth noting is that there are red blood corpuscles in the protoplasm between these vacuoles (vide the left-hand side of the figure). The nuclei are oval-shaped, and the chromatin is very peculiarly arranged, its outward appearance resembling the shape of a chrysanthemum. In the center there is a nucleolus, and it must be noted that a nuclear condition of this kind is generally very rare.

In figure 12 the protoplasm is glutted with numberless vacuoles, and it is for this reason remarkably foamy in appearance. The vacuoles are of two sizes, of which the smaller ones are mostly located in the deeper portion and make a somewhat continuous layer, though in other parts there are to be found some of these smaller-sized vacuoles also. The larger vacuoles are crowded together in the middle part of the layer, and some of them burst forth onto the surface, while others make their appearance in the innermost layer close to the Langhans' cells. The plastosomes are quite uniform in shape with those illustrated in the preceding figure, and they are all found in the protoplasm between the vacuoles. The lipid granules are nearly of the same size and are found at several places, some of them with the halos distinctly described. The nuclei appear to be somewhat smaller in size, but there are no remarkable changes in their structure.

These structural conditions in the syncytium layer which I have illustrated and described above can be detected at almost any time and place at the different periods from the first month of pregnancy to nearly the fourth, and, therefore, there is no doubt that these structural changes cannot be taken as a measure to tell the precise time of pregnancy. At no time after the fourth month of pregnancy can we detect the plastosomes. The lipid granules and vacuoles reach their maximum growth from the second to third month of pregnancy, and after the fourth month they gradually begin to decrease, entirely disappearing after the seventh month. The syncytium layer comes in sight in a

remarkable manner on the seventeenth or eighteenth day after pregnancy, reaches the maximum of growth at the end of the first month, and from the second to the third month it seems, although not very conspicuously, more or less reduced in thickness, but after the fourth month it suddenly becomes thinner, and simultaneously its structure becomes simplified, and in the seventh month it will altogether atrophy and remain simply in the form of a thin membrane like an endothelium. Moreover, in the last two months the layer will disappear in some places and will be noticeable only as a discontinuous thin cover. In other words, this layer, excepting in the early stage of pregnancy, always decays and goes out of existence in inverse proportion to the growth of the embryo, and this is the very point which should engage the careful consideration of those who are interested in the functions of this layer.

## *2. The Langhans' cells (figs. 13 to 26)*

The Langhans' cells have in general distinctly clear bodies, and are distinctly bordered with a thin membrane (pericula?) on the surface. Their sizes, shapes, and structures are extremely varied; on examination of the smallest cells (figs. 13 to 16 and 7, 8 and 10) it will be found that they are either round like a ball or oval-shaped, with foamy nuclei of corresponding shapes inside. The structures of the cell bodies consist of quite structureless stroma and a large quantity of plastosomes, of which the latter are rod-shaped in several lengths, and are usually distributed all over the cells, though sometimes they crowd together on one side of the cells. There is occasionally a small oval-shaped body somewhat dark in color close to one side of the nucleus, which might possibly belong to Meves' so-called 'Centrotheca,' it, however, lacks a centriole within (fig. 13). Within the nuclei there are generally one or two conspicuous nucleoli, and in most of the cases it is difficult to discern the nuclear network. In the large sized cell bodies (figs. 17 to 19 and 2, 3, 9, 11 and 12) we always find either a small quantity of lipid granules or vacuoles. The lipid granules are nearly the same in size, and, though small in number, they are scattered

everywhere (figs. 17 and 11). The vacuoles are extremely varied in their size, quantity, and arrangement, and it is for this reason that the structures of the cell bodies have so many special features (figs. 18, 2, 3, and 9). In such vacuolar cells the rod-shaped plastosomes are for the most part short in length, and they are arranged either along the walls of the vacuoles or crowded together in the protoplasm between the vacuoles; however, sometimes it will be found that, as will be seen in figure 18, the vacuoles are chiefly placed in order on the outskirts of the cells, and the plastosomes accumulate in the center around the nucleus. Again, it will be found that, as in figures 19 and 12, both the lipid granules and vacuoles of various sizes are simultaneously contained in the cell bodies, in which case the plastosomes are comparatively small in number and are scattered here and there in the protoplasm between the lipid granules and vacuoles.

In the largest cells (figs. 20 and 21) the cell bodies are in general well filled with a great number of vacuoles of various sizes, and consequently the protoplasm is noticeable only around the nucleus and between the vacuoles. There are almost no lipid granules, which, when present, are small and are very few in number; also the plastosomes decrease in quantity and are mostly found around the nucleus.

In short, the smaller-sized cells have in general only the plastosomes as their material components, while the larger-sized ones still contain a number of lipid granules and vacuoles and in the largest ones the cell bodies are commonly vacuolated in a high degree and the protoplasm decreases considerably in quantity, the plastosomes in general gradually decreasing in number as the cells grow in size, though it sometimes happens that it is very difficult to demonstrate them, regardless of the size of the cell bodies. Of figures 22 to 26 it will be observed that figure 22 shows the lipid granules only, figure 23 chiefly the small vacuoles and a few lipid granules; in figure 24 it is entirely the same as the former, however, with the distinctive feature that the vacuoles are remarkably large and have lipid

granules of various sizes within; in figure 25 and 26 the bodies are vacuolated to the highest degree, and it is perfectly plain that the vacuoles which are extremely varied in size and shape mix together and grow larger, thus giving the cell bodies the appearance of a honey comb in a striking manner. The large and highly vacuolated cells such as are illustrated in figures 21, 25, and 26 are very frequently met with in the Langhans' islets.

The various structural images in the Langhans' cells that I have described above make their appearance in a most remarkable manner from the end of the first month of pregnancy to the end of the third month, and in the fourth month, though the plastosomes are still existent in a remarkable degree, the lipid granules and vacuolar formations are no longer conspicuous, and in the following months not only do the cells decrease suddenly, but also these materials component of the cell bodies disappear, though the cells in the Langhans' islets retain those structures as long as they exist.

### 3. *The stroma cells of villi (figs. 27 to 38)*

The smallest of the stroma cells of villi, as is illustrated by figure 27, are mostly ball-shaped and have the nucleus of a similar shape within. In the cell bodies there are plastosomes, mostly rod-shaped. Figures 28 and 29 are nearly the same as figure 27 in their shape, though the one contains in the cell body a somewhat large quantity of lipid granules of different sizes, while the other has a small quantity of extremely small lipid granules and an equally small quantity of small vacuoles. Figures 30 to 38 illustrate the cells arranged in their approximate order of size and, though their shape and structure appear extremely varied at a glance, it will be found on close examination that, with the exception of figure 34, there is a structure which is common to nearly all the rest, the only difference between them being principally the size and number of vacuoles contained in the cell bodies. Generally speaking, the larger sized-cells have vacuoles which are naturally large in size and number, and the fact that large vacuoles are built up to some extent from

the fusion of the smaller vacuoles which are connected with one another can be often proved in these stroma cells (fig. 38). The plastosomes are mostly rod-shaped and lie scattered in the protoplasm located between the vacuoles, though they sometimes crowd together in a somewhat larger number in certain places (figs. 31, 32, 36, and 38). The lipoid granules are generally few in number and are found between the vacuoles, though at times they are present within the small vacuoles (fig. 36). The smallest of these lipoid granules, at a glance, bears a close resemblance to the granular plastosomes; however, since they are mostly very distinctly bordered, besides being stained darker, it is easy to distinguish them from the former (figs. 36, 37, and 31). Figure 34 looks somewhat different from the various cells described above in that the cell is nearly spherical, with a remarkably large nucleus within, besides a small number of somewhat large vacuoles and plenty of lipoid granules in the cell body. These granules have various sizes, but are in general of middle size and a few of them are distinctly included in the vacuoles. The plastosomes are extremely limited in number, and lie scattered in the protoplasm between the vacuoles. It is very seldom that this kind of cells makes its appearance, and a great majority of cells in the stroma, as are chiefly illustrated by figures 35 to 38, present a distinct vacuolar formation.

The plastosomes in the stroma cells have in general a very strong staining power, and are therefore more easily detected than other cell groups. It is, moreover, very rare that the cells which have no plastosomes are met with, but in stroma cells the lipoid granules seldom appear. The stroma cells appear distinctly and are therefore most easily found during the period from the second to sixth or seventh month of pregnancy. In the eighth month, usually, numberless capillary blood vessels suddenly grow and increase within the villi, so that it is impossible to examine the cells. With every possible method it was difficult to detect the cells, and, therefore, I am inclined to believe that the stroma cells suddenly cease to exist at this stage of pregnancy.

#### 4. *The decidual cells (figs. 39 to 71)*

It is a generally well-known fact that decidual cells are divided into very many kinds according to shape, size, staining properties, and structure; however, it has not as yet been definitely decided whether or not these kinds of cells should be reckoned as one and the same sort. Marschand ('04) first divided the decidual cells into two types according to the difference in size. Subsequently, Fraenkel ('14), too, who studied them chiefly from the staining standpoint, set up a similar theory, and tried to divide them into his so-called acid cells (Eckersche Form) and the neutral cells (of large type); however, he himself and others had no doubt that there was not only no distinct division between these two kinds of cells, but rather there was existent an intermediate type of cells between them.

Figures 39 to 69 illustrate the various kinds of decidual cells placed in order. Of these, cells such as in figure 39 are the smallest and are spherical with a nucleus of a corresponding form. The protoplasm is, as compared with the interstitial cells at the time of non-pregnancy, remarkably large in quantity, and contains in it a large number of rod-shaped plastosomes. Figures 40 and 41 are a little bit larger than the former, and the one is spherical while the other is oval, both having a nucleus of nearly corresponding shapes. The protoplasm becomes still more abundant and the plastosomes are somewhat longer rods. It is worth our noting that both cells have a kind of boraer membrane already on the superficial layer of the cell bodies. Figure 42 demonstrates the first appearance of a few lipid granules of various sizes within the cell bodies. Figure 43 illustrates a pear-shaped cell, which holds in the body a somewhat large quantity of granules and a few vacuoles. A few plastosomes are to be found, and they make their appearance only on one side of the cell. Figure 44 resembles the former in shape somewhat, and contains in the cell body remarkably large lipid granules, which, on a close examination, are found to have a more or less distinctly clear halo around each of them, as though they constituted the contents of vacuoles. The plas-



tosomes are mostly rod-shaped, and they crowd together on one side of the nucleus, while on the other they appear in a remarkably long, granular string (Fadenkörner). In figures 45 to 48 the cells have exceedingly varied shapes, and the nucleus trends toward one side of the cell close to its superficial layer. The cell bodies are filled with numerous irregular-sized vacuoles, which present a more or less distinct foamy structure. The plastosomes are mostly short and rod-shaped and they are to be found in the proplasm between the vacuoles. Some of them are arranged in a long row along the walls of the vacuoles, while others are found in groups in a certain section. The lipid granules are, in general, small in number, and their sizes are irregular, some of them finding themselves distinctly at the center of the vacuoles (e.g., fig. 46).

In the various cells described above it will be observed that the nuclei are generally dark-colored, with indistinct nuclear network in most of the cases, though the nucleoli contained are conspicuous enough. Figure 49 is extremely different in its appearance from these cells. The cell body presents a vacuolar formation in high degree and the protoplasm proper can be demonstrated in a small amount only along the surface of the nucleus, at which place only a few rod-shaped plastosomes are found. The border membrane on the surface of the cell is very distinct and the nucleus is different from that in the various cells described previously in that it is clear and presents a large foamy body. The nuclear network is somewhat distinct, and, besides, there are conspicuous nucleoli. It must be generally stated that this kind of cell appears very seldom. In figure 50 the cell body presents an equally high vacuolar formation as in the former, and, in addition to that, the vacuolar walls entirely disappear in some places and so allow the inner spaces of the vacuoles to communicate with one another, thus clearly indicating the evidence of the vacuoles having been agglutinated. A few more plastosomes than in the former are found in groups in these cells, and, besides, there is a small quantity of lipid granules, mostly within the vacuoles. What is especially peculiar about this cell is that at the center of the cell body there appears a black-

colored homogeneous star-shaped lump, from the surface of which are shot forth a number of processes, which run over directly to the protoplasm between the vacuoles. The proper nucleus is hardly detected. In figure 51 the cell is longish, and the overabundance of plastosomes which are distributed densely almost all over the cell body is the feature of this cell. Between there, is a somewhat large number of vacuoles of various sizes, and no lipid granules at all are to be found.

In figures 52 to 54 the various cells illustrated are gradually larger than those described above, the cell bodies are filled with a large number of vacuoles and granules. The latter are extremely irregular in size and density and are sometimes found as contents of the former. The plastosomes are mostly short rods, and especially in figure 52 they are somewhat abundant, and some are found massed along one side of the nucleus. In figures 53 and 54 they are comparatively fewer and lie scattered between the vacuoles. The thin layer on the surface of the cell is thicker and more distinct in this kind of cell, to such an extent that it almost reminds us of the ordinary cell membrane. In figure 53, as in figure 50, we notice a black-colored round-shaped lump at the location of the nucleus; however, in this case, the surface of the lump is smooth and has no process. Comparatively few cells have such a black-colored lump, and, as in these cells it is always difficult to tell the whereabouts of a nucleus of normal condition, I am quite at a loss to know whether or not the dark lump described above should be deemed a modification of the nucleus or regarded as that part of the protoplasm which is just adjacent to the nucleus which has, by reason of its staining properties, obscured the nucleus. This question, along with the stained lumps in the syncytium layer as illustrated in figure 10, constitutes a puzzle, and I have mentioned it here for the sake of future investigations. However, in view of the fact that numerous plastosomes, which are the important elements of a living cell, are always demonstrated in the cells concerned, while at the same time they present no noticeable regressive phenomena. I am rather inclined to believe that it is possible to attribute a certain functional significance to these unknown lumps.

The cells illustrated in figures 55 to 57 are already exceedingly large, and they are, at a glance, recognized as Marschand's so-called large-type of decidual cells. On the surface there is a rather thick layer, which may be divided into two of which the inner one is thick and the outer thin, and they exactly remind us of the definite cell membrane. The cell bodies consist of plastosomes, lipoid granules, and structureless stroma. The plastosomes are mostly long rods or threads, some being more or less peculiarly curved and the quantity of plastosomes is variable. The lipoids differ also in point of size, density, quantity, etc. What is worth our noting is that there is no vacuolar formation to be found in these cells. The nuclei are large, clear, and foamy. The nuclear network is especially conspicuous in figures 56 and 57. Figures 58 to 61 show the definite form commonly belonging to the so-called decidual cells of large type. In these cells the cell membrane is remarkably thick, and the distinction between the inner and outer layers is always clear. What deserves our special attention at this juncture is that the outer layer contains, in most cases, a kind of granular body which is somewhat large and yet irregular in size and stained a dark color. The cell bodies consist of a large quantity of plastosomes and homogeneous stroma. The plastosomes are mostly long rods, and they sometimes appear extremely elongated in the shape of threads (fig. 61). They are distributed equally all over the cell bodies, although they sometimes tend to appear more or less in groups. The plastosomes in these large cells have, in general, very slow staining properties, and, therefore, they are a very difficult subject to be dealt with from the technical point of view. The nuclei are foamy and dark-colored, and the nuclear networks are indistinct.

The cells illustrated in figures 62 to 69 differ from those described above, and they all lack the plastosomes. Even in the most excellent stained preparations these cells appear within the decidual tissues in small numbers, for the most part more or less in groups, scattered like islets, so that it is, as a matter of course, incomprehensible that here alone the plastosomes are hard to be demonstrated. However, as, in consideration of

their structure and shape, it is premature yet to decide positively that there is a tendency for a retrogressive change among all these cells, I will here suggest provisionally that such a phenomenon is a sign of a certain functional period in the cells concerned. Now, at first, in figures 62 and 63 the cell bodies are comparatively dark, and within they contain a large quantity of vacuoles and lipid granules of various colors; some of the vacuoles distinctly have a lipid granule as their nucleus, while others, being placed in rows close to the cell membrane, present a peculiar image. The nuclei are clear and have a somewhat distinct nuclear network. In figure 64 the cell body is filled with numberless vacuoles of nearly the same size, and on one side of the nucleus accumulates a large quantity of protoplasm, and, besides, there are a few deep yellowish-brown lipid granules. Figure 65 is of nearly the same type as the former, but the lipid granules contained are by far greater in quantity than the vacuoles. The nucleus is as clear as the former, with conspicuous nuclear network. Figures 66 and 67 illustrate the cells whose bodies are filled with an exceedingly large quantity of vacuoles of various sizes, in consequence of which the protoplasm becomes comparatively scarce and faint and is mostly noticeable only around the nucleus. And, besides, there are some vacuoles which hold dark or deep yellowish-brown lipid granules; also vacuoles and lipoids, whose size, quantity, and distribution are as varied as will be seen illustrated in the respective figures. The nuclei are generally dark and show extremely delicate network formations.

Figures 42 to 48 and 51 may be compared, from their sizes and histological point of view, with that class of cells which is termed by many authors as 'decidual cells of small type' (or possibly Ecker's type), while, on the contrary, figures 55 to 61 and 64 to 69 may be nothing but the so called 'large-type' or ordinary decidual cells (neutral cells). Further, the various cells illustrated by figures 49, 50, 52, 53, 54, 62, and 63, judged from their size and internal structure, should be deemed an intermediate type which may intervene between the former two, since it is a very difficult task to determine to which one it

should belong. Now, the result of my careful examination of the appearance and distribution of these cells as compared with the time of pregnancy has been that, in the material which was taken a fortnight after conception, this being the earliest I have on hand, the cellular ingredients of the decidua chiefly consist of the small-type cells described above. In a little more advanced stage (i.e., about 17 or 18 days after pregnancy) the decidua shows also the appearance of a large quantity of the 'intermediate-type cells,' while in the few days following (i.e., about 22 or 23 days after pregnancy) with the decidua already of definite growth, all kinds of cells, especially the large-type ones, can be detected in comparatively large quantities. One month after pregnancy the large and intermediate type cells appear as the principal ingredients of the decidua, while on the contrary the small-type cells retrograde gradually and are met with only in the interstitial tissue. Such condition is maintained until the end of the last month of pregnancy. In short, I conclude from the histological and histogenetical point of view, that the various kinds of cells described above all belong to one class, and consequently it follows that the division of decidual cells into large and small types, is faulty. In other words, the term 'small-type decidual cells' applies only to the cells which are still at the early stage of growth, while the 'large-type cells' are those in the last stage of growth. The time taken in such growth is, according to my observations at the earliest stage of pregnancy at least, comparatively small, thus the small-type cells being perfected into the 'definite large-type cells' in so short a time.

The appearance of lipoid granules and vacuoles in the decidual cells is most remarkable from the end of first month to the second month of pregnancy, and they gradually decrease in the months following, though very infrequently they can be demonstrated even at the end of pregnancy. The plastosomes could no longer be demonstrated in any of the decidual cells after the seventh month of pregnancy.

And, in the interstitium of the decidua, such extremely strange-looking productions as are illustrated by figure 70 may sometimes be detected. They are stained dark and consist mostly

of a great number of granular bodies which are extremely varied in size. There are granular threads, which are the result of the granules being linked together, more or less curved large rod-shaped bodies of different lengths, and sometimes threads which are very long and often curved like screws, besides a large number of material ingredients, which, being mixed up among them, have shapes similar to them and yet are unstained and noticeable only as a shadow. The former, which are susceptible to staining, are dyed deep red by Altmann's method and deep black by iron-alum-haematoxylin. At a glance they look like plastosomes in their shape and staining, and yet in general excel the latter in size considerably. If aggregated, they may be quite easily detected under medium magnification. Moreover, the staining properties of the ingredients are much stronger than the plastosomes, and they bear a rather close resemblance to lipoids in their density and appearance. The product of this kind do not only possess exactly the same properties in shape and stain as the granular bodies in the cell membrane of large-type decidual cells to which I alluded above, but also indicate that there is often the closest relation between the two; i.e., within the cell membrane of the cells concerned there are, besides the granular bodies above referred to, often short granular threads or large rod-shaped bodies, both of which are similar to the substances in the interstitium described above. One end of the rod-shaped bodies sometimes enters deep into the cell membrane and swells into a club-like shape, while a greater part of the other end juts out into the interstitium, thus giving itself the appearance of passing into the interstitial product. I am not able to explain the original nature and functional significance of this kind of product, and yet, according to the afore-mentioned observations, I have no doubt whatever that in the formation of the product the large cell, and especially its cell membrane, plays an important part and, since such interstitial substances are demonstrated in large quantities in the adjacent blood vessels as are illustrated by figure 71, it may be inferred that they are ultimately absorbed in the vascular organs. The products of this kind are for the first time noticed at about the

second month of pregnancy, appear most conspicuously from the end of the second month to the third, decreasing gradually after that, and, though the decrease is considerable after the fifth month, they may yet be demonstrated until about the sixth month. Besides the above, there are detected in certain parts of the interstitium numberless filar productions which have various length, and are sometimes long like threads or fibers, of which the smaller and shorter ones sometimes bear a close resemblance to the plastosomes, while the others usually gather in great number and often make a mass of fibrous bundles. This kind of product, so far as staining is concerned, is entirely similar to the interstitial productions described above, and yet it differs from the latter in that its shape is not so varied, its thickness is nearly always even, and, besides there is no special relation which is noticeable as existing between the products and neighboring cells.

5. *The epithelium of the uterine gland (figs. 72 to 83)*

As is generally well known, the uterine gland undergoes a certain morphological change at the early stage of pregnancy, and in my previous treatise I have drawn attention especially to the fact that the glandular cells also show a morphological change at such a time. Here I will observe and describe more thoroughly the changes of the cells concerned.

Figure 72 shows a glandular cell which is commonly noticed at the early stage of pregnancy and which is already remarkably thickened and somewhat round. The shape of the nucleus for the most part corresponds to that of the cell. On top of the cell there are traces of cilia. The cell body contains many slender and rod-shaped plastosomes, which latter chiefly gather closely against and surround the nucleus. In figures 73 and 74 the cell grows larger, and the plastosomes are demonstrated only in the upper half, while in the lower half which contains the nucleus, none of them are found. At this section of the cell there are plenty of lipoid granules, which are of about equal size and are stained a bright yellowish-brown color, and on top of both cells there are still the traces of the cilia. In figure

75 the cell is remarkably elongated, and within are contained a great number of lipid granules. The nucleus is oval with a nuclear network distinctly observed; from this period on no more traces of cilia are to be found. The various cells illustrated by figures 76 to 78 gradually increase in their size and, since their swelling is remarkable, especially in the upper half, it is usual that this part of the cell naturally juts far out into the lumen, though the lower half, being comparatively narrow, is closely united with the basement membrane. Within the cell body are contained a great number of both lipid granules and vacuoles, of which the vacuoles in figure 76 are as yet small and few and they chiefly occupy positions in the upper half of the cell body, whereas in figures 77 and 78 the vacuoles enlarge tremendously and fully occupy the upper half, in consequence of which cell bodies have the appearance of a honey comb in a high degree, and the protoplasm remains only as a thin wall which separates the vacuoles. The lipid granules in the latter two kinds of cells chiefly crowd together at the base of the cell bodies, and only a small portion of them are left behind as contents of the vacuoles. The afore-mentioned three cells each present conspicuous nuclear network and nucleoli. And in the various cells in figures 75 to 78 no plastosomes are to be detected.

Figure 79 illustrates a large oval-shaped cell, which has a similar-shaped large alveolar nucleus. The cell body, because of the vacuolar formations of various sizes, presents the image of a conspicuous honey comb, while the plastosomes lie scattered somewhat plentifully in the interstice between the vacuoles. Though there are some extremely small vacuoles in jet black, no ordinary lipid granules in coarse grains are to be found. The contents of the nucleus are nearly homogeneous, and the characteristic nuclear network is not found, but within the nucleus there are two nucleoli. Figure 80 is an extremely irregular-shaped cell, with the nucleus of a corresponding shape. The structure of the cell body is nearly the same as that in the preceding figure, and the vacuoles are somewhat plentiful, but the plastosomes are very few, and, besides, there



are but few yellowish lipid granules. The nucleus has a conspicuous network.

As the change of the glandular cells reaches a high degree, the cells leave the basement membrane in large numbers and are isolated in the glandular lumen. Such cells I call temporarily the 'desquamated cells' in contrast to the cells which continue to settle on the basement membrane or remain fixed on the walls. Figures 81 to 83 illustrate my so-called desquamated cells, in the inside of whose cell bodies there are many vacuoles and a small number of extremely small lipid granules which are stained in black. At a glance they look like the 'wall-fixed' cells, and yet on a careful comparison we find that there is a more or less remarkable difference between them. That is to say, in the desquamated cells the cell bodies are in general somewhat turbid, and also the vacuoles look somewhat withered and decayed. No plastosomes are to be found, and, besides, the change of the nucleus is remarkable, as will be seen in figure 81. Here it is converted into a dark-colored and homogeneous lump provided with a very irregular contour, having within a few small nucleole-like bodies. In figure 82, as before, the nucleus is simply a homogeneous and dark-colored lump; in figure 83 it has swollen somewhat remarkably, and the nuclear network is extraordinarily conspicuous. In short, the desquamated cells have undoubtedly begun a retrogressive degeneration already, and from the existence of many broken pieces of cells which are always found in the glandular lumen it can be simultaneously demonstrated that the desquamated cells are doomed to break up and perish at this section sooner or later. The various changes undergone by the glandular cells described above are seen in a remarkable degree already on about the seventeenth or eighteenth day of pregnancy in the decidua serotina, while in the decidua vera it is a little bit later and the changes are noticed to about the same degree as the former only toward the end of the first month of pregnancy. Both reach the maximal changes at about the end of the first month of pregnancy, and on the days following they gradually pass into the so-called desquamated cells and break up and perish as such.

The series of changes described above have a more or less difference of time between the decidua serotina and the decidua vera, viz., in the former they may be followed up vigorously up to the end of the second month of pregnancy, though in the third month they suddenly decrease, whereas in the latter such changes may be demonstrated even one month later.

THE HISTOLOGICAL STRUCTURES AND THEIR FUNCTIONAL  
SIGNIFICANCE (SOME REFLECTIONS ON LITERATURE)

In the preceding chapter I have given a somewhat minute account of the delicate histological structures of the various important cell groups which exist in the placenta and the decidua vera at different periods of pregnancy. Now, on a perusal of the observations given therein, it will be found that as components which are common to the various cell groups there are 1) plastosomes, 2) lipoid granules, and 3) vacuoles. As a matter of course the degree of the appearance of the three kinds of components and their distribution in which they are present vary infinitely as the kind of cells differs or according to each individual cell. However, that which exercises the most important influence over the shape and formation of the cell is chiefly the lipoid granules and vacuoles, of which the latter often appear in a very great number and occupy the whole body of the cell, thus giving the cell a highly foamy appearance or a honey comb structure. The cell which has fallen into such highly vacuolar formations makes one feel, at a glance, that it has presented a phenomenon of collapse due to the regression of the cell concerned, as some observers are apt to conclude quite hastily. However, as, on a careful examination of it, it is found that, in spite of the high degree of changes shown by the cell, there are demonstrated for the most part within the cell body the plastosomes which are deemed an important active element in the functions, and also in consideration of the normal structure of the nucleus and of the fully stained conditions of the cell body, there is no doubt as to the cells being alive. And even if it should be conceded for a while that the cell having the fully stained vacuoles as described above is the indication of a kind

of regression, who could explain the reason why this kind of 'regressive' cell actually appears in so high a degree within the tissues of the placenta and the decidua at a certain period of pregnancy, and especially in the first half of pregnancy when the tissues should grow in a most vigorous manner? Therefore, I am confident that not only is it wrong to deem such vacuolar formations a death phenomenon of the cell, but also they should rather be taken for a quite significant phenomenon which shows a certain function of the cell concerned. And, as regards the actual existence of such a function, I am inclined to assert from their closest resemblance to the structures of many other glandular cells, as a result of my histological observations, that a secretory function is existent in these kinds of cells. However, as the problem is of such a provisional character I will, for the present, reject all hasty assertions, but instead will consult literature widely and make general reference to the previous interpretations of many authors on the structures and secretory processes of the various glandular cells in the organs in which secretory functions are definitely known, so that the most deliberate considerations can be given my histological observations and their functional significance, which, being compared and discussed under impartial criticism, it is hoped, will help toward making the original nature of the functions clear.

Since the relations between glandular histology and secretory functions were early dwelt upon by R. Heidenhain ('68, '75, '80) with his penetrating eyes, the subject has aroused the interest of many excellent physiologists and histologists, and the studies of the subject have since followed so quickly one after the other, that it would be difficult to enumerate them here. The following are the principal authors who have studied the subject, and the materials chosen by them for investigations were chiefly pancreas, salivary glands, gastric glands, lacrimal glands, skin glands, and pelvic glands, all of which are usually known as representative glands in many kinds of animals classed above the amphibians: Schultze, Fr. E., '64 and '67; Langhans, '69; Pflüger, '71; Schwalbe, '71; Ebner, '73; Nussbaum, '78 to '82;

Ebstein and Grützner, '74; Lavdowsky, '76; Langley, '79 to '89; Mathews, '80; Klein, '82; Biedermann, '82 and '86; Flemming, '82; Kühne and Lea, '82; Nicoglu, '93; Altmann, '94; Galeotti, '95; Krause, '95 and '97; Müller, '96 and '98; Solger, '96; Zimmermann, '98; Held, '99; Maximow, '01; Noll, '01; Fleischer, '04; Heidenhain, '07; Babkin, Rubaschkin, and Ssawitsch, '09.

I will not go to the trouble of giving a detailed account of each of the results of these research works, but will confine myself to summarising the main points of their investigations respecting the structure and secretory phenomena of glandular cells, which are most essential to my studies, and refer to the original works for details.

In the first place, the structure of the glandular cells having a duct, or externally secreting glands, greatly differs, as is generally well known, according as they are serous or mucous. The serous cells have an exceedingly large number of granular bodies, and consequently their characteristic is that they are generally dark. These granular bodies are generally known as Cl. Bernard's secreting granules. The relation which the latter has to secreting functions has been generally recognized by many an interesting research work since that of R. Heidenhain, and it will be noted that Heidenhain, having observed a kind of slender thread-like structure which exists close to the basement membrane, of the glandular cell of a dog's pancreas, for the first time drew general attention to a peculiar sort of organic structure which is existent in the glandular cell. This kind of thread-like structure was demonstrated also in the salivary ducts and convoluted uriniferous tubules in after years, and it cannot be anything but M. Heidenhain's so-called 'Basal-filamente' or our plastosomes.

Below I wish to give a general outline of the changes appearing in these organic tissue elements of the glandular cells which will follow the secretory functions.

Now, at first, it is universally agreed by every author that secretory granules increase or decrease according to secretory functions. According to the result of the close examination with respect to such correlation, conducted chiefly while in a fresh

condition, of the pancreas of the rabbit, the oesophagus glands of the frog, and the gastric glands of the water lizard, by Langley ('79 and '89), whose exposition of the correlation is best authenticated, it will be noted that during suspense of their functions glandular cells are generally glutted with secretory granules, whereas as secretion begins the granules gradually decrease and disappear, in consequence of which in each cell will appear a sharp demarcation between the homogeneous wide outer layer and the remaining granular inner layer. This observation of Langley has been repeatedly proved by many other authors in many other glands, and its validity except in a small number of cases, has been recognized by nearly everybody.

For convenience, I defer to a later section a minute explanation of the functional significance of the thread-like production which is found in the basal part of glandular cells.

Even in mucous cells it has been universally acknowledged by many authors since F. E. Schultze ('64) that there are granular bodies in large numbers at a fixed period of secretion, and at the time when secretion is very high these granules gradually disappear as in the serous cells (Langley, '79; Biedermann, '82). It seems therefore that in the forms of secretion formation mucous cells agree, for the most part with the serous cells. However, the reason why both differ widely in their structure is that in the latter the secretion is, for the most part, speedily drained into the glandular lumen at the time of secretory function, whereas in the mucous cells it is formed within the cell bodies, besides being stored up there for a certain period, thus giving the cells a peculiar structure and a characteristic clearness.

In short, in the aforementioned two kinds of glandular cells, the large quantity of secretory granules always to be seen in both during suspense of secreting functions disappears by degrees as the functioning begins. In view of this fact, we have no doubt whatever that, at the time of secretion, the granules always play an indispensable part as the mother-ground for the secretions and, since secretory granules are generally deemed a solid production, according to the investigations of many authors

while the secretions are mostly fluid, it follows that it would be no great error to take it that, viewed simply from the histological standpoint, the so-called secretion, after all, means, the liquefaction of secretory granules. However, the modes of liquefaction are, according to my view, so varied that they should by no means be dealt with in one and the same manner, but rather there are, roughly speaking, several forms, such as are given below, according to the kinds of glandular cells, or according as the cause which prompts secretion differs.

*First form.* This is observed in certain mucous cells. The secretion is brought into being simply by the melting down and growth of secretory granules which have developed in a fixed degree, and, different from many other glandular cells in which the secretory granules are mostly preproducts of secretion, the granules here in this case show the same reaction as secretion (that is, mucin) from the beginning of their appearance and find themselves already identical with the secretion as early as they appear. This fact was demonstrated by M. Heidenhain in the goblet cells of the intestine of the salamander, and it is, in general, difficult to tell definitely the time of liquefaction in what is covered by this form.

*Second form.* This is observed in many mucous cells. The granules, while in the first stage, appear as a certain preproduct (mucigen), and undergo a chemical change simultaneously as they have developed to a certain degree, and are changed into the ordinary secretion (i.e., mucin). Mention has been made to this effect by Biedermann (the mucous cells of the frog); M. Heidenhain and Nicoglu, '93 and '98 (the skin glands of a salamander); Altmann, '94 (the submaxillary glands of a cat); Maximow, '01 (Glandula retrolingualis of a hedgehog).

*Third form.* This is often seen in ordinary serous cells. As the functions begin, the secretory granules begin to liquefy gradually on the periphery and, although it appears that the granules are imbedded for a certain period in the secretion already formed, they finally change thoroughly into secretion, and then appear as simple vacuoles within the cell bodies. The clear halos around the granules referred to by Langley ('34),

Corlier ('96) and Maximow ('01) were it seems, deemed by these authors an essential ingredient in the formation of glandular cells; however, M. Heidenhain with Nicolas ('92) attributed one part of them rather to artificial production, while the other was brought into being in the form it appears in, possibly because the granules were reduced in size in connection with secreting functions. And in recent times the researches regarding pancreas cells, conducted by Babkin, Rubaschkin, and Ssawitsch ('09), have proved the above mentioned views of Heidenhain with more force and accuracy; that is, in the opinions of these authors, clear halos around the granules are, after all, nothing else but secreted matter which appears as a liquefied product of the granular substance. And that such an image should be a sign of an important period in secretion formation within the cell bodies will be clear in the statement made by the three authors mentioned above on the changes which zymogene granules undergo at the period of a pancreas secretion: "Wir erhalten den Eindruck, als ob das (Zymogene) Körnchen sich allmählich auflöst, in dem es sich immer mehr und mehr verkleinert und in ein kleines verwandelt" (p. 92).

*Fourth form.* According to the views of Babkin, Rubaschkin, and Ssawitsch above referred to, this presents an appearance somewhat like a modification of the third form. That is to say, many secretory granules together with the intergranular substance concerned form themselves into somewhat large masses, which latter slowly begin to liquefy from the periphery, and are then gradually changed entirely into secretion in rather large drops, to be drained out into the glandular lumen. Compared with the third form, in which each individual granule becomes liquefied separately, this type differs from the former in that a number of granules coagulated together in lumps are transformed gradually into drops of secretion. This form is distinctly demonstrated at a time when the function of pancreas cells is very greatly increased, especially by means of stimulating the vagus nerves or of soap infusion in the duodenum, it being characteristic of the secretion at such a time that the latter is very dense, and is rich in albumen and fer-

ments. And the three authors mentioned above are of the opinion that the several bodies which are revealed by this secreting form may be compared with the small bodies which are often known as 'Nebenkerne,' 'parasome,' 'corpusculus paranuclaires,' and 'noyau accessoir' in the pancreas cells of the lower vertebrates.

*Fifth form.* This points to a case in which the substance of secretory granules changes its quality at a certain period, becomes soluble, and is only dissolved in the watery part of the secretion alone, as was instanced by Babkin, Rubaschkin, and Ssawitsch, in the experiment of the pancreas secretion after pouring acid in the duodenum. As a proof thereof, the secretion always shows in this case the same coloring réaction as do the zymogene granules.

The three forms (the third to the fifth) described above are together met with in the pancreas cells, and it deserves special attention that according as the cause differs for the rise of secretion, even in one and the same glandular organ, there is a difference in the forms of secretion, and consequently in the nature of the secretion produced.

*Sixth form.* Certain secretory granules sometimes have two extreme developments. As an instance, M. Heidenhain observed the skin glands of a salamander and he found that, as the development of the granule reaches a certain degree, one part of it is liquefied and passes into a viscous secretion, while the other goes on growing in size, and is perfected into the characteristic poison grains.

*Seventh form.* A certain organic structure is presented in the secreting granules; at the beginning it is not different from ordinary cases, and yet as a certain development is accomplished, every granule is divided into a crescent-shaped section (Kapuze) which is stainable, and into an unstainable section (Träger) within the crescent-shaped section, thus forming the 'Halbmondkörperchen' so termed by M. Heidenhain. This latter as it grows in size is considerably enlarged, especially in the Träger, and consequently the Kapuze is flattened gradually and is only stuck like a plate on its one side. Then the Träger



is at last dissolved altogether and is changed into secretion, while at the same time the Kapuze is also condensed, and forms itself into secondary granules, later on to be drained out along with the secretion, thus bringing about an entirely granule free condition of the cells. This form of secretion was first detected and examined closely by M. Heidenhain in the pelvic gland of a salamander and in the lacrimal gland. Subsequently, Nicolas ('92), in a man's lacrimal gland, Held ('99), in a rabbit's sub-maxillary gland, and Fleischer ('04), in a cow's lacrimal gland, observed and proved the appearance of the crescent-shaped small bodies described above.

The above does not cover all the forms of granular liquefaction at the time of glandular secretion, and yet it will be quite clear how varied the modes of liquefaction are.

Now, I will discuss a little further the origin, and therefore the manner in which supply is made of secretory granules, this problem being the second in importance with respect to glandular secretion.

With regard to the origin of secretory granules, the problem itself is a very difficult one indeed, but it should be noted that, along with the sudden increase of studies on plastosomes in recent years, many authors have attempted a solution of this troublesome question.

Below I give a general account of the result of these researches. Generally speaking, every author equally agrees in the argument that every glandular cell always has within the cell body plastosomes as constant ingredients and, though the quantity and arrangement of the latter are certainly varied, it is the usual condition that while the serous cells are in a state of rest the numerous plastosomes are chiefly arranged in rows along the long axis of the cells. These plastosomes, however, generally decrease in quantity considerably during the period of secretion, especially when the cells are filled, and they are then found largely, close to the basement membrane of the cell, while near the lumen, they are either entirely absent or, if they are found, they are between the granules in a very small number. And in mucous cells, in contrast to serous cells, the plastosomes are found largely

near the nucleus, though some lie scattered in the protoplasm between the secretory granules, all being arranged in an irregular order. With regard to the functional significance of the plastosomes contained in these glandular cells, there are various theories, such as the theory of secreting mechanism by Benda ('03, p. 780), that of water-secreting apparatus by M. Heidenhain ('07), and that of prop system by Bruntz ('08); however, these are either the historic ones, having been refuted experimentally by Meves and Regaud ('08) already, or mere assumptions.

What is believed by a great majority of authors at present is that plastosomes are very closely related to the formation of secretory granules. And the first man who published his views with regard to this was Altmann ('94), who thought that his so-called 'vegetative Fäden'—a greater part of which agrees with our plastosomes of to-day—being split up in small pieces are formed into granular bodies in large numbers and make the beginning of secretory granules. Quite recently the researches of Laguesse ('99 a, b, '05, '11), Regaud ('09), Regaud and Mawas ('09), Hoven ('10, '11, '12), Champy ('11), Schultze ('11), have followed one after the other, and, though their observations may differ a little from one another, either in trifling points or in the form of description, they none the less fall entirely into line with Altmann's view that the formation of secretory granules has its origin in plastosomes. And, moreover, the fact that, as is generally acknowledged, the number of plastosomes in glandular cells always increases or decreases according to the changes of secreting functions is nothing if not forcibly proving the validity of such a theory.

However, this very theory is not without objections. M. Heidenhain, Mislowsky ('11), and Levi ('12) are those who are opposed to it. Especially M. Heidenhain, taking his stand on the 'Protomeren-Theorie' which he set forth, states that secretory granules should have their origin in extremely faint and extraordinarily small bodies in the protoplasm which are beyond our sight; these bodies growing up and increasing should gradually develop into the smallest granules—his

'Primärgranula'—which are seen microscopically to gradually bring about the growth of definite secretory granules. Thus it seems he denies the histogenetical correlation between plastosomes and secretory granules. His theory, though most dexterously proposed on the most profound proofs, is after all a mere hypothesis. Besides, in consideration of the following quotation, it will be quite clear how he holds the views that his so-called 'Primärgranula' have a close relation with the genuine cytomicrosomes in their formation, and that the latter do correspond to a section of his 'Basalfilamente,' and, further, that the filaments are identical with Flemming's Mitom:

Ich bin daher mit Solger der Meinung, dass auch in den serösen Drüsenzellen die Basalfilamente Teil eines Fadensystems sind, welches nach der Bezeichnungsweise Flemming's dem sogenannten Cytomitom zugehören würde. . . . Nicht ganz ausschliessen darf man jedoch zur Zeit die Möglichkeit, dass die Drüsengranula eventuell von den genuinen Plasmamikrosomen sich ableiten, welche nach unseren allgemeinen Erfahrungen immer innerhalb der Protoplasmafilamente liegen, also verdichtete Teile feiner Fädchen sind. . . . Da nun die Körperchen beiderlei Art (Primärgranulis und genuine Mikrosomen) morphologisch schwierig unterscheidbar sind, so kann eine verwandtschaftliche Beziehung zwischen beiden zur Zeit wenigstens nicht abgelehnt werden, besonders da wir über die positive Bedeutung der genuinen Plasmamikrosomen noch sehr im Unklaren sind (p. 390).

And since it has already been clearly proved by the researches of Meves ('07) that Flemming's Cytomitom agrees with our plastosomes, it would appear that M. Heidenhain does not only maintain the view that his 'Basalfilamente' exists as a water-secreting apparatus as mentioned above, but at the same time also supports, instead of disproving, the argument which regards basal filaments as being the matrix for the formation of secretory granules, as Altmann and others do. As regards Mislavsky's view, it will be noted that he, in line with Levi, takes the ground that as between plastosomes and secretory granules there were no conspicuous conditions to be detected in support of the existence of a formative relation between the two. However, anent Mislavsky, it will be noted that the invalidity of such a view has been exhaustively pointed out by a Belgian cytologist, Duesberg, in comments noted for their profundity

and thoroughness. The following phrase is there employed by him: "Ein negatives Resultat beweist nichts gegen eine Reihe positiver Resultat und man kann nur schliessen, dass Mislawsky die Bilder nicht beobachtet hat, die seine Gegner unter Augen hatten" (p. 785). This should incidentally prove to be a pertinent comment on the theory of the excellent Italian author, Levi.

If we summarize the observations of the various authors respecting the structures and functions of the glandular cells described above, we may enumerate as constituents of the glandular cells, 1) plastosomes, 2) secretory granules, and, 3) secretions (vacuoles), besides the protoplasmic stroma proper, and it would be superfluous to state that of these constituents secretory granules have a directly important relation to the glandular secreting functions. The secretions are nothing else than a liquefaction or modified product of the latter and there is no alternative but to assume that the secretory granules, once lost at secretion, take their matrix mostly from the plastosomes, which latter, being split up and separated in small pieces, gradually meet the deficit so caused. Thus, the real state of glandular secreting functions is already a thing which can be largely followed up and made clear by means of minute histological investigations to-day.

Also the histological studies of the ductless glands—internally secreting cells—have become very active of late years, and, especially among those which have been carried on by the methods of plastosomic study, we may enumerate the thyreoid and parathyreoid glands, suprarenal capsules (chiefly its cortex), Langerhans' islets of the pancreas, and the ovary. Of these organs, the ovary has been used more often than the rest as the object of study, and consequently, comparatively speaking, much is known about it. I will therefore give a general summary of the observations of the various investigators respecting this organ, consider its structure and the relations of its internal secretory functions, and then glance at the structures of the other organs, thus contributing toward a histological account of internal secretion in general.

That the corpus luteum is a kind of internally secreting organ (from the histological point of view) was advocated by Prenan as early as 1898 and by Born in 1900. Subsequently, the argument has been advanced with more certainty by a close histological study conducted by Regaud and Policard ('01), Fr. Cohn ('03), Mulon ('09), Athias ('11), Van der Stricht ('12), Tsukaguchi ('12, '13), and Levi ('13). And the main ground of this argument lies in nothing but that the luteal cells contain as formative ingredients of the cell bodies plastosomes and lipid granules, and often demonstrate a very large quantity of vacuoles which may be deemed their secreted matter, and also that the close correlation between these ingredients functionally is plainly apparent in the same manner as it is seen in external secretory cells. Of the various ingredients mentioned above, lipid granules certainly have the most important significance in internal secretion; however, since the latter is not only characteristic of luteal cells, but is also widespread among the other kinds of internal secreting cells, Tsukaguchi has already argued that it would be proper, in view of its functions, rather to compare them with the secreting granules of ordinary glandular cells.

In the next place, Cohn first regarded the vacuoles in a rabbit as a secreted matter of the luteal cell, and he saw them simply as a dissolved product of lipoids; subsequently Tsukaguchi also studied the same animal, and likewise deemed them a modified product of lipoids. However, vacuoles differ exceedingly in the degree of their development in all kinds of animals. For instance, Van der Stricht did not notice any vacuoles in the luteal cell of a bat, and subsequently Levi's observations regarding the same animal nearly agreed with those of Van der Stricht. In the guinea-pig, however, Levi noticed a small number of vacuoles, which he attributed to a retrogressive phenomenon of the cell concerned, and he stated that this phenomenon, by means of a chemical change of lipoids following it, could cause possibly the substance of the latter to be dissolved by the benzol and xylol which had been used by him as clearing media. In short, whenever the vacuoles

appear, the lipid granules first make their appearance as their forerunners, and the former pass into the latter in succession, as has been entirely agreed upon by many observers.

With regard to the origin of lipid granules, the Zoyas ('91) pointed out that there was a certain quantitative relation between granules and plastosomes, and this fact was acknowledged by Mulon, Athias, and Tsukaguchi, though Levi alone tried to deny it, as in the case of external secretory granules.

With regard to the secreting phenomena of luteal cells, Van der Stricht argued that in the order Chiroptera it was possible to divide them into two forms, viz., serous secretion and lipid secretion. By serous secretion is meant that the follicular epithelium, being the antecedent of the luteal cells, secretes liquor folliculi, and, according to him, the Graafian follicle, after rupturing, still keeps on secreting in this manner for a fixed period, it being characteristic of this secretion that in the peripheral part of protoplasm of the young luteal cell some serous infiltration appears, and for that reason gives that part generally a somewhat transparent appearance. Already at this period a large quantity of small lipid granules appears within the cell bodies; however, since these granules do not as yet perform any functions, he called this stage the serous secreting period of the luteal cells; then, as the ovum settles, the corpus luteum has already reached its highest degree of development, and he termed it the second stage of corpus luteum formation.

At this stage the cell body is already filled with numberless lipid granules, which, undergoing a chemical change, cause the cell body to keep on discharging secreted matter until the end of pregnancy. This is what the lipid secretion means. This theory seems to have been afterward accepted in the main also by Levi. However, the condition is entirely different in the Rodentia, and especially in the rabbit. According to the observations of Cohn and Tsukaguchi, lipoids chiefly appear in a high degree only in the early part of pregnancy, then disappear rather speedily. In the second half of pregnancy the lipoids change into vacuoles almost as transparent as water, the cell bodies present a highly distinctive vacuolar structure, such condition being

still more conspicuous toward the last period of pregnancy. Now comparing this with what is observed in the Chiroptera mentioned above, this period of lipoid secretion, so-called by Van der Stricht, in the rabbit passes away in a comparatively short time, and then slowly passes to the stage of the characteristic vacuolar image; thus, it appears, it presents a certain stage of secretion which is peculiar to itself. In short, it is interesting to note that, in consideration of the changes in the forms of secretion in external secretory cells, the structure, and therefore the form in the secreting functions even in the same luteal cells, differ according as the class of animals differs.

That the interstitial cells of the ovary possess the same inter-nally secreting functions as luteal cells, by reason of the close resemblance which they bear to the latter in shape and structure, has been universally acknowledged in the researches made, in various kinds of animals, including the Rodentia, Chiroptera, and Carnivora, by Regaud and Policard ('01), Limon ('02, '03), Fr. Cohn ('03), Regaud and Dubreuil ('06), Mulon ('11), Athias ('11, '12), Van der Sticht ('12), Tsukaguchi ('12, '13), Levi ('13). Now, according to these observers, the interstitial cells also contain, as do the luteal cells and many other glandular cells, important constituents, such as plastosomes, lipoid granules, and vacuoles. The vacuoles are especially very plentiful, and they, for the most part, present a minute, delicate, and peculiar vacuolar image, the protoplasm proper being barely noticeable around the nucleus. The lipoid granules, as compared with the luteal cells, are generally small in quantity and, moreover, it is sometimes difficult to detect them. It is customary for the lipoid granules more or less to increase in quantity at the time of pregnancy. It has been equally acknowledged by many observers that the lipoids of the interstitial cells generally appear for a comparatively short period, commonly fade away in color and change in quality speedily, thus gradually passing into the vacuolar substance. And then, many authors, except Levi, have proved and recognized, even in this case, that plastosomes have a direct relation in the formation of lipoids.

Entering into details, Mulon stated that in the rabbit the plastosomes, granular in form at first, change into minute siderophil granules, and then diffuse siderophil substance, and the lipoids will be formed from the latter. Athias ('12), who examined a newborn bat, also seconded the argument of Mulon for the most part, and argued that plastosomes produce the lipid first at their center and accumulate it there, and in support of his argument he stated that occasionally the cortex, which has the same staining properties as plastosomes, could be detected around the lipid granules. Tsukaguchi certified to an intermediate type of granules between the plastosomes and the lipid in the young interstitial cells, thus arguing that the granular plastosomes develop and grow in size directly into the lipid granules, as are seen in the case of the luteal cells. In short, the various investigators have not as yet come to an agreement in their views as to the correlations between the two, and yet it has been universally acknowledged by them that as the cells grow and increase and the lipoids or vacuoles appear plentifully, the plastosomes decrease in quantity in inverse ratio.

With regard to the organs other than the ovary, Mulon ('10 a, b) closely examined the suprarenal capsules of a guinea-pig and rabbit, and stated that by the conglutination of the granular plastosomes was produced directly a substance (possibly our lipoids) which has affinity for osmic acid (osmophil) or iron-alum-haematoxylin (siderophil), and is introductory to the formation of a vacuolar secreting matter to follow. Celestino da Costa ('07), Champy ('09), and Colson ('10), also having experimented on the cortical cells of the suprarenal of a cat, guinea-pig, toad (*Bombinator*) and bat, have proved the same fact as above. Bobeau ('11) also argued that when the parathyreoid glandular cells of a horse form a certain effective product the plastosomes should play an important part. And, besides, Engel ('09), Mawas ('11), and Schultze ('11), each demonstrated the plastosomes in the thyreoid and parathyreoid glands of a man, a rabbit, and a frog, and, according to Duesberg, it was stated that the plastosomes could be detected in the Lanhans' cells



of the pancreas. It should none the less be stated that a majority of the facts enumerated above are merely preliminary. Many future investigations must be looked forward to for a further enlightenment of the secretion of these organs.

In summarizing the histological knowledge we have at present of the internal secretory cells described above, almost every cell has, as its constant ingredients, plastosomes, lipoids, and vacuoles. Now, the plastosomes are not regular either in their shape or arrangement while the lipoids are not quite regular in their size, quantity, and color, but the smaller ones bear a resemblance to granular plastosomes, while the larger ones, agglutinating with one another, form larger fatty droplets. The contents of the vacuoles probably consist of watery, transparent droplets, which are separated from one another by a very thin partition wall. The cell body should present a more or less conspicuous alveolar structure in proportion to the quantity of vacuoles contained. The quantitative correlation by which these formative ingredients are connected to one another is not free from considerable variations. Occasionally only one or two of them exist to the absolute exclusion of all the rest. It must be very often the case that such phenomenon may be partly due to the difference in the order of animals chosen for the subject of study and partly to the functional relation of the cell concerned. In short, it may be said that the various formative ingredients described above as being seen in internal secretory cells constitute equally necessary constant elements, the same as with external secretory cells, as has been minutely dwelt upon previously. Now, it is a marvelous sight indeed to compare histologically these two kinds of cells, and to look at the perfect agreement, not only in their structures, but also in the various histological changes which follow their functions. On this score, I am convinced that should there be a structure like the two kinds of cells described above, besides the functional changes which very nearly correspond to the above, it would be no error to bring such cells under the category of glandular cells, regardless of the existence of a duct in them. And, if we look at the

various cells of the placenta and the decidua, we will find that all of them are well furnished with various conditions which mark the glandular cells above referred to, and that naturally we should assert the existence of secretory processes in them also. In the following section, I will give a detailed account of the secretory phenomena of the various cell groups.

#### THE PHENOMENA OF INTERNAL SECRETION IN VARIOUS CELLS OF THE PLACENTA AND DECIDUA

It is too plain to need argument that in all cases the real state of the life processes of any cell cannot be made clear unless all the phenomena in the living condition of the cell concerned be followed up closely with the microscope. However, since it is certainly difficult to attain such an object by the histological method employed by us at the present time, by noting the phenomena of internal secretion it is possible to denote each of the extremely varied structural images obtained from the preparations fixed and stained as indicating a certain period in the phenomena of secretion; to compare carefully and consider the correlations between the different periods, and thus to infer the whole of the process of secretion. Therefore, I must ask the reader to take this point into consideration.

##### *1. The phenomena of internal secretion in the syncytium layer*

Now, at first, in figures 1 and 2 only plastosomes are present, and lipid granules and vacuoles are entirely absent, so that we may conclude that this shows the early stage at which secretion is not yet in appearance or when secretion is at rest. In the next place, in figures 3 and 4, more or less lipoids appear, and vacuoles are either barely found at one part or not formed as yet; the lipoids, in general first appear on the superficial layer, at which place they tend to grow up and increase gradually. The plastosomes have decreased considerably in quantity especially in figure 4. The last two figures may be taken as the early stage of secretion in the syncytium layer, and as it passed to the subsequent stage, a large number of vacuoles, viz., secre-

tions, appear besides the lipoids. The vacuoles later on keep increasing, while, on the contrary, the lipoids decrease in quantity, and, moreover, of these vacuoles those which are near one another unite into vacuoles of various sizes, and it will be seen that the surface vacuoles of the latter ultimately rupture and open upon the surface of the syncytium layer. The process of secretion described above may be followed in figures 5 to 7. Should this process continue and reach its highest degree, such structural images as are shown in figure 8 would be probably brought about in the end! What is peculiar in these various stages when secretion is very high is that plastosomes are detected. In the next place, what is shown in figure 9 is taken, similarly to what is in figures 3 and 4, for a comparatively early stage of secretion. However, the former differs more or less from the latter in that already a somewhat large quantity of vacuoles is noticeable in it. And, especially, the vacuoles are chiefly arranged close to the Langhans' cells. Figure 10 demonstrates on one side a large quantity of plastosomes and on the other a region where no plastosomes are to be found. This region presents, as mention has been made already, a foamy structure which can be detected only by careful attention. Figures 11 and 12 each clearly demonstrate plastosomes, besides a large quantity of vacuoles and a small quantity of lipoids. That is to say, in figures 9 to 12 it is always easy to demonstrate plastosomes at different periods of secretion, and therefore the various periods of secretion shown therein may be differentiated from those given in figures 5 to 8, though it is not easy to decide what correlations these stages have periodically between themselves in point of secreting functions. However, according to the various images described above, it is deemed practicable in the main to arrive at the following presumptions with respect to the phenomena of secretion of this cell layer:

*a.* At a time when function of secretion has not yet begun, the chief ingredient of this layer is plastosomes, which are found in a very large quantity; however, as the function begins, they suddenly decrease in quantity, and at a certain period,

especially when lipid granules are exceedingly plentiful, they entirely disappear. It has already been explained how this lack of plastosomes is not the result of the want of skill in the making of preparations.

*b.* As the function begins, lipid granules first appear conspicuously, especially at a place which is close to the superficial layer, and then vacuoles appear. Sometimes both can appear simultaneously at a comparatively early stage (fig. 9).

*c.* The direct relations between the lipid formation and plastosomes could not be ascertained in my preparations. However, the origin of lipid granules was found in an extremely small granular body, and on the other hand it is clear that there is a tendency for the plastosomes either to decrease more or less in quantity as function of secretion increases or to entirely disappear.

*d.* The transparent halos which are sometimes found around the lipoids give the latter an appearance of being the contents of vacuoles, and by reason of such a condition we are led to believe that a certain intimate relation exists between lipoids and the vacuolar formation (figs. 7, 9, and 12).

*e.* However, we cannot as yet positively state whether or not all vacuoles without exception have an intimate relation with lipid granules such as is described above. For instance, I cannot definitely declare whether the formation of the extremely delicate foamy image such as is seen in a part of figures 8, 10, and 12 has been the result of the lipid granules while in the earliest stage of lipid formation, that is, while as yet in a stage in which they are exceedingly small, having changed their quality, and caused such small vacuoles to grow in groups, or whether as Mulon quoted before, has observed with respect to the ovarian interstitial cell, at the period of his 'diffused siderophil substance' (though it is still beyond my power to prove that such a period could appear in the syncytium layer), the substance has, instead of forming lipoids directly assumed a vacuolar formation. It is for future investigations to solve such a question.

f. All vacuoles gradually unite, and it appears that they possibly make a kind of canal system running irregularly and crosswise within the syncytium layer, and some of them distinctly open their mouths in various places on the surface of the syncytium layer, thus it is apparent that their contents, their secretions, are drained out into the maternal vessel of the intervillous spaces. And, as is seen in figure 11, the blood corpuscles which are noticeable within the syncytium layer may be rightly taken for the mother's corpuscles which have accidentally gone into the canal system mentioned above, which would in turn prove the existence of the latter.

g. The secreting function is at work from the beginning of pregnancy to the end of the fourth month, though it is most active in the second and third months.

## *2. The phenomena of secretion of the Langhans' cells*

The protoplasm in the Langhans' cells which are still small and should be deemed comparatively young contains plastosomes only (figs. 13 to 16); however, as the cells reach a certain size the lipid granules appear (figs. 4, 11, 12, 17, and 22). The latter have developed from extremely small granular bodies such as are visible in figures 9 and 21, and their number is not very large; the vacuoles appear in a very conspicuous manner, and it seems that these have also grown into what they are comparatively speedily from a very minute form, it being clear that the larger of them have been brought into being by the agglutination and joining together of some of the vacuoles. And, while the secreting process is in progress, it seems that lipoids play a directly essential part; how they change their quality and liquefy from the periphery, and thus gradually pass into vacuoles as in the syncytium layer, can be very clearly seen in figures 12, 23, and 24. The loss of lipoids which keeps going incessantly by such vacuolation is made good by fresh formations elsewhere, and thus, the same process being repeated, the vacuoles go on growing in number and size simultaneously as the cell appears more and more to increase in its capacity,

though the preparations I have are still very poor to prove this positively. However, the fact that, as mentioned above, lipoids have their origin in very small granular bodies and that plastosomes considerably decrease in quantity as the cell grows in size and therefore its functions are promoted (please refer to figs. 13 to 21), cannot but be taken for having proved that plastosomes, owing to their quantitative relations, take part in the lipoid formation and, if this deduction be practicable, it would follow that the large group of plastosomes which makes its appearance in an especially limited section, as is seen in figures 3 and 9, should not be without significance for the new growth and supply of lipoids. In short, the phenomena of secretion of Langhans' cells, in general, are not very active and yet the cells in the Langhans' islets are somewhat different, and it seems the functions are very active in this part, so much so as to make it a feature of these cells that they present a very large and highly vacuolar formation (figs. 21, 22, and 26).

Since the Langhans' cells always have on the surface a comparatively conspicuous border membrane, there is no alternative for the contents of vacuoles, viz., secretions, but to pass through this membrane and be drained into the villous tissues, and they are therefore possibly bound to be ultimately absorbed on the side of the embryo. However, since the part like the Langhans' islets where the functions are necessarily very active is, as is well known either mostly wrapped up in the decidual tissue or exists within the intervillous spaces, floating directly in the mother's blood, it is possible that the secretions coming from such a place are absorbed on the mother's side. Moreover, the large number of vacuoles which is found in a part where the Langhans' cell layer comes in touch with the syncytium cell layer as in figures 9 and 12, judged from the position they occupy, has been temporarily denoted by me as forming secretions of the syncytium layer and so described in the previous paragraph; however, I am afraid nobody can say for certain that it is so. If we suppose that the secretions are brought forth by the Langhans' cells, who may say they will

not come out into the intervillous spaces together with those of the syncytium layer? In a word, since the Langhans' layer is entirely closed against the mother's body by the syncytium layer in the early stage of pregnancy, it would follow that the secretions are entirely in the service of the embryo, but after that it is probable that a part of them are taken also by the mother's body.

The function of secretion of the Langhans' cells, just like the syncytium layer, is active in the main almost from the first stage to the end of the fourth month of pregnancy, though in the second and third months it is very active, suddenly subsiding with the fifth month. In the Langhans' islet it continues still longer and commonly gradually subsides after the sixth or seventh month.

The epithelium of villi is located between the circulation of the mother's body and that of the embryo, and it is for this reason presumed that it must be an organ which takes nutrition for the embryo, as has been commonly held in literature, but that such is a groundless assumption must be quite clear from my histological observations given above. And, besides, there are some important reasons which prove the utter fallacy of this theory. It is in the first to third months of pregnancy that the growth of the epithelium of villi is most active. If the functions of the alimentary organ for the embryo be assigned to it, the epithelium of the villi should go on developing most vigorously, but the fact is quite the reverse, and it retrogrades and becomes thin in the second half of pregnancy, and accordingly the decline of functions is brought about. This is one of the absurdities. And in the eighth month of pregnancy, when the embryo calls for a still greater increase in the supply of its nutrition the capillary blood vessels of villi increases suddenly, as was mentioned above, and early in this stage the epithelium of villi becomes remarkably regressive and falls into decay, so that the embryonal circulation of the villi is separated from that of the mother only by a thin membrane like endothelium, undoubtedly it being quite easy for both to allow the interchange of materials between them. In other words, the

particular organ which is needed for the absorption of nutrition for the embryo first makes its appearance in a perfect condition only after the epithelium of villi retrogrades and becomes thin. On this score I am led to believe that the epithelium of villi is simply an organ of internal secretion, and that the ground is extremely weak for the argument, which treats it as an organ to take nutrition for the embryo, as has been generally conjectured in the past.

### *3. The phenomena of internal secretion in the stroma cells of villi*

The smallest of the stroma cells of villi is simply a ball-shaped cell which is comparatively rich in protoplasm, and within the cell body there is a large quantity of plastosomes (fig. 27), but presently a somewhat large quantity of lipoid granules or vacuoles having various sizes appears within the cell body (figs. 28 and 29); and subsequently, as the cell grows in size the chief ingredients of the cell body will be plastosomes and vacuoles, while the appearance of the lipoids is not very distinct. The image such as is seen in figure 34 is very seldom met with. On the contrary, however, the vacuoles may be deemed the almost constant ingredients of each cell, and especially as the cell develops and grows in size they increase the more in size and quantity, and present a highly foamy structure which is characteristic of this kind of cell. Now, if we consider the correlations between the different constituents mentioned above, it will be found first that in these cells the plastosomes are stained comparatively easily, and are therefore very distinctly detected in each cell; and as regards its quantitative relations, it will be noted that there is not the least tendency in the plastosomes to decrease in quantity, even though the functions increase and the cells enlarge, as was seen in the epithelium of villi described above. On the contrary, the plastosomes crowd together in large numbers in the various protoplasmic sections of cell bodies, and they present the appearance of a new growth and multiplication in the sections concerned (figs. 30, 31, 32, 33, 36, and 38). The only exception is that when the quantity of lipoids contained in the cell



body is remarkably large, the plastosomes decrease more or less remarkably in quantity (figs. 28 and 34). The lipid granules, as mentioned above, do not appear in very large numbers and, since they arise from very small granular bodies, it is very difficult to clearly discriminate the latter from the ordinary granular plastosomes (figs. 28, 29, 31, 36, and 37). Therefore, in consideration of this fact and of the quantitative relations between lipoids and plastosomes as described above, I am inclined to trace the mother-ground of the lipid formation in the plastosomes. Then, with regard to the vacuolar formation, we may infer from the conspicuous halos which often appear around the lipoids, or from a phenomenon in which the lipid often occupies the position of a nucleus within the vacuole (figs. 29, 34, and 36), as in the case of the epithelium of villi as described above, that the vacuole should of necessity be the liquefied product of a lipid. In short, it may be stated that the secreting phenomena of these cells, if looked at from the histological view-point, are very simple indeed, and plastosomes first bring forth lipoids, which latter in turn change into vacuoles, and the reason why the lipoids are comparatively scant is that the period of their appearance is exceedingly short. And, while perhaps on one hand the contents of vacuoles, viz., secretions, are gradually drained out of the cell body, on the other the protoplasm and therefore the plastosomes bring about a prospective new growth and multiplication, presumably to provide for the materials of the next secretion, and in this manner the afore-mentioned process, as simply it may be, is repeated and follows in succession. At different times and in different places, to make a secondary or tertiary secreting process within a cell all the time, the cell develops and grows in size gradually, and its structure therefore becomes extremely complicated, and in this way I suppose that, even in one and the same cell body, the various periods of the phenomena of secretion make a simultaneous appearance according to the ingredients contained. This is a mere hypothesis of mine, and yet since it was early refuted by M. Heidenhain that the secretory granules of all kinds start their individual function

separately as a small independent 'organel' within the cell, this hypothesis of mine should not be taken exception to. And moreover, the increase and mass of protoplasm or plastosomes and the lipoids at different phases, all of which could be demonstrated in every part of this cell at any time, if sought for an interpretation of their significance, will each provide a material to substantiate the hypothesis mentioned above. In this manner, this cell, while promoting its secreting functions on one hand, grows in size more and more, and such like relations could be recognized more or less even in the Langhans' cell.

The functions of secretion in the stroma cells of villi begin at the end of the first month of pregnancy and continue actively until about the seventh month, though they are most active from the second month to the sixth. And though in the eighth month it appears that they suddenly subside, it will be found at all times that it is difficult clearly to follow the destiny of each of the cells, since it is interfered with by the strong increase of the capillary blood vessels of villi at this stage, as mentioned already. Since no special duct was detected, it is difficult to tell how the secretions are removed, other than by attributing it to osmose, as in the case of the Langhans' cells, and by predestining the secretions to be absorbed by the embryo.

#### *4. The phenomena of internal secretion of decidual cells*

If we first look at the decidual cells of the smaller type (figs. 39 to 51), we find that the chief components of the cell body in the youngest are plastosomes (figs. 39 to 41), next appear lipid granules (figs. 42, 43, and 44), then follow vacuoles, it thus being customary for the great majority of decidual cells of smaller type to contain many vacuoles and more or less lipoids besides plastosomes. The plastosomes sometimes decrease more or less in quantity in inverse ratio to the lipoids or vacuoles (figs. 43 and 49), but more often is it difficult to discern such relation, and, besides, many are plastosomes which either form a conspicuous group in some part of the cell body or considerably

increase in quantity (figs. 44, 48, 50, 51, and 52). The lipoid granules arise at the beginning in a very small granular body, whence they grow up to a certain degree, when clear halos appear around them, and the manner in which they directly participate in the formation of vacuoles (figs. 44, 46, 52, and 53) is the same as what is observed in the epithelium and the stroma cells of villi. And sometimes it occurs that the vacuolar formation appears equally at a time within one and the same cell body, and as a result the foamy image of high degree, such as is illustrated by figure 49, is brought into being, but this is rather rare, and in most cases the vacuoles vary in their sizes. And, besides, it is customary for the vacuolar formation in most cases to contain at the same time groups of plastosomes or lipoids of different sizes. In short, the smaller-type decidual cells entirely agree with the stroma cells of villi in their structure, and, therefore, there is no need for argument that their secreting phenomena should be dealt with in the same manner as the latter. On this score, I will not go to the redundant trouble of touching upon the secreting process of the smaller type decidual cells here, but will confine myself to the brief statement that the function is repeatedly performed by the same methods as in the stroma cells of villi.

If we take a glance at the figures in the plate, it will be quite clear that the smaller-typed decidual cells, repeatedly performing as they do the functions as described above, develop and increase in size more and more; and passing through the various intermediate types (figs. 52 to 54, 62 and 63) gradually, as I mentioned in the previous chapter, pass into the larger-type decidual cells to attain the height of their growth. Therefore, the demarcation between the large and small types in the decidual cells is, after all, due to the difference in the degree of growth of the same kind of cells, and the smallness of the cell should be taken for an indication of comparative infancy, while the largeness of the cell shows that it has attained the region of perfection in its growth.

The large-type decidual cells may be divided into two kinds with respect to structure. One represents the kind of cells whose

body is protoplasmic, and commonly has a large number of plastosomes, besides more or less lipoids which are often discernible, though vacuoles are almost absent (figs. 55 to 61). The other represents those cells whose body presents a highly vacuolar image, whereas the protoplasm considerably decreases except around the nucleus, while no plastosomes are to be found. The lipoids contained are irregular in their quantity, but more or less of them are always existent (figs. 64 to 69). A great majority of the commonly so-called decidual cells belong to the former, while a comparatively small number is represented by the latter. In the former class the structure of the cell is entirely different from the small-type decidual cells and my 'intermediate type,' so that along lines of histology there is no indication of the existence of the process of secretion, and although lipoids exist in small numbers, their quantity quickly decreases and they go out of existence as the cell body grows up in size, so that it would be in order to denote the lipoids rather as persistent bodies bequeathed from a period of their growth, and consequently it follows that it would be no great error to conclude that at this period a secretion, such as was noticeable at the period that preceded it, either considerably declines or entirely disappears. However, in the various cells which belong to the latter class, the afore-mentioned secretory functions are developed to the extreme throughout all their growth, and there is an appearance which points to the utter exhaustion of plastosomes on account of these functions. From the scantiness of materials, it is difficult to determine the destiny of this kind of cells; whether the cell body ultimately breaks up and decays or is absorbed or whether after throwing out the secretions, the plastosomes again increase or are replenished, and thus it slowly passes into the former class of cells; however, I at least am confident that it would be premature to assert that the various periods illustrated stand for a direct indication of retrogression or decay. In short, in the larger-typed decidual cells, it is possible clearly to observe in a portion of them the same process of secretion as in the small

type cells, whereas in the largest number there is almost no sign of such a function, which fact is worth much attention.

The large-type decidual cells, as aforesaid, no longer present the ordinary phenomena of secretion for the most part, and yet, at a certain period, dark-stained coarse granular bodies of irregular sizes often make their appearance within the bordering membrane of the periphery (figs. 55, 59 to 61); various material products having the same staining properties are often detected within the interstitium (figs. 70 and 71), which makes one feel that there is a certain formative relation between the two. And, besides, similar products often filling up the blood vessels around them, give the appearance of being absorbed in the vascular organs (fig. 71). Such peculiar products having been originally observed in the fixed preparations, it follows that they might be an artificial product, a result of the fixatives, and yet from the observations mentioned above it is not difficult to conclude that a certain material which corresponds to them is prepared, perhaps by some special function of the cell membrane of the cell concerned, and is sent forth in the direction of the interstitium. And should this supposition prove correct, it would follow that these two kinds of large-type decidual cells are functionally quite independent of one another, though they are genetically of the same origin.

Looking on the whole of the functions of the decidual cells from the histological point of view given above, I am led to believe that they may be roughly divided into three periods, according to the course of their development. The first period is seen in all the small-typed decidual cells, the intermediate type so termed by me, and in a portion of the large-typed cells, here the secreting functions are distinctly performed in the same manner as in the stroma cells of villi. In the second period, possibly by the functions of the cell membrane, a certain product is prepared, to be sent forth in the direction of the interstitium. The third period begins after the sixth month of pregnancy, when the cell body in general shrinks considerably, and no plastosomes are to be discovered, besides no particular tissue structures from which inference may be made of the functions performed are to be recognized. And, moreover, cells

at this period experience the rise of embryonal pressure (the inner pressure of the uterus) as the time of pregnancy elapses, in consequence of which they are remarkably flattened and afterwards present the appearance of flattened epithelium. And, as regards the destiny of decidual cells, it seems that it has been argued in the past that they retrograde and perish by fatty degeneration or coagulative necrosis (Klein); however, as a matter of fact, I have not as yet discovered such a change. All kinds of decidual cells perfect their growth comparatively rapidly early in the beginning of pregnancy, viz., in about three weeks after pregnancy, and after that only a quantitative increase or decrease of the various cells occurs. Consequently, the degree of growth of the cells cannot be the sole measure of the time of pregnancy. However, judging from their quantitative relations, it is not difficult to arrive by way of inference at the approximate period of pregnancy; that is to say, the small type cells appear from about the second week of pregnancy to the end of the first month, and the intermediate-type cells from above the seventeenth or eighteenth day to the end of the second month, in both cases in exceedingly large numbers, while the large type cells appear throughout the whole remaining period beginning about the twenty-second or twenty-third day of pregnancy, and yet it will be noted that these large cells are at the height of their activity during the period from the end of the first month to the end of the third month of pregnancy, and while in the fourth month the functions are still pretty high, they considerably decline in the months to follow, and in the seventh month and after it is very seldom that such functions are clearly noticeable.

The phenomena of secretion of cells in the decidua serotina in the first half of pregnancy are nearly the same as in the decidua vera as described above. In the second half, especially after the eighth month, giant-cells grow in large numbers, and somewhat remarkable changes take place, even histologically in the ordinary sense of the term, and, therefore, I have examined the subject with an especially keen interest; however, the absence of good materials, coupled with the difficulty in staining them has hindered me in making excellent preparations,

and the functions of cells at this period are therefore set aside for future investigations.

5. *The phenomena of secretion in the uterine glandular cells at the time of pregnancy*

The epithelium of the uterine gland undergoes a remarkable change in the early part of pregnancy, viz., on about the seventeenth or eighteenth day after conception (figs. 72 to 83). Now, if we consider its phenomena of secretion, we shall find that, even in this cell, lipid granules first appear, and then vacuoles are formed. In the stained preparations, lipid granules appear assembled and are accumulated especially near the basal part of the cell body and show a remarkably clear yellowish-brown color (figs. 73 to 78). The latter often appearing as contents of vacuoles (figs. 77 and 78), it would probably appear that the vacuoles are a modified product of the lipoids, just the same as in the other cases. In this way the lipoids gradually change into vacuoles, the cell grows in size and presents a highly honeycomb structure (figs. 77 to 80). The plastosomes either decrease in quantity or become very difficult to discover as the functions of secretion increase in activity. However, I have not been able to make clear the formative relations between the plastosomes and lipid granules. Be that as it may, it happens that, with the increase of the function of secretion and the growth of the cells, the latter gradually move over toward the comparatively enlarged glandular lumen, and, at last, leaving the wall of the glandular tubule, are entirely free within the glandular lumen. The characteristics of these desquamated cells are that either the cell body shows a highly vacuolar formation or that the vacuoles being somewhat reduced in quantity, the protoplasm becomes dark and turbid, and no plastosomes are to be found. The condition of the nucleus is also exceedingly abnormal (figs. 81 to 83). How the various cells of this kind are broken up by degrees and added to the large quantity of fragments filling up the glandular lumen can be observed and followed with a great certainty.

The various changes of the uterine glandular cells as described

above have, as was mentioned in my own observations, a definite relation to the time of pregnancy, and accordingly the rise and fall of the functions of secretion of these cells also act upon it; that is to say that, in the first month and the first half of the second month of pregnancy, the functions are at the height of their activity, and they subside considerably from the beginning of the third month, the subsidence being by far the greater in the fourth month, and in the fifth month they seem to come to a standstill, it being no longer possible to demonstrate the function of secretion in the months that follow, viz., in the second half of pregnancy. In the next place, the aforementioned functions of the glandular cells, as compared with the decidua vera, appear more speedily and in a still higher degree in the decidua serotina, and fail accordingly earlier than in the former, and everybody easily recognizes that the secretions of the glandular cell and its broken-up matter both accumulate in the glandular lumen for a certain period, though some consideration should be given the question as to how they are removed or absorbed. It is said that, according to what has been written on this subject, the placental formation commences from the second month and is perfected in the fourth month and that the decidua reflexa and decidua vera are agglutinated in the fifth month. Should this opinion be true, it would follow that the secretions of the uterus, looked at from the periodic relations of secretion, are for the most part drained into the uterine cavity, and take a part in the formation of the so-called uterine milk. However, according to my own experience, it appears that the placental formation and the adhesion of the decidua reflexa take part in an earlier part of pregnancy. Therefore, I am inclined to believe that a part of the secretions and detritus of the uterine glands, at least in a little advanced period of pregnancy, are naturally absorbed by the mother on account of the closure of the ducts.

#### SUMMARY

It is to be observed that the syncytium layer, Langhans' cells, stroma cells of villi, decidual cells, and uterine glandular cells, all of which constitute the chief tissue elements of the placenta



and decidua, each contained plastosomes as a constant ingredient of its protoplasm, and that a majority have at the same time a certain quantity of either lipid granules or vacuoles, or of both, and, consequently the minutes histological structure of these cell groups bears a close resemblance to that of both the internally and externally secreting cells. And, moreover, these main components of protoplasm or cell body are, according to their functions, as closely correlated to one another as they are in the glandular cells. Now, taking a general survey of this correlation, it was found in my study that the plastosomes, being the first constituent, appear for the most to be the matrix of lipid granules from which the latter rise, and as a proof of this argument, I will cite the stroma cells of villi, in which the correlation between the two is very closely shown. We can notice it somewhat in the Langhans' and decidual cells, and if we closely examine the manner in which the lipoids appear in these cells, it will be found that they always rise from granular bodies which are very small and strongly siderophil. I believe that these may be rightly compared with the so-called 'Primargranulis' which Heidenhain found in the common glandular cells, and even though they appear very small, they do appear as a perceptible body. There is no evidence to be found of their appearing as slowly growing and increasing, as Heidenhain assumes to be the case, from an infinitely small body which is hardly seen microscopically until they enter the vision of a microscope. Rather is it found that some of them bear a close resemblance to the granular plastosomes in their size and staining properties, clearly indicative of images running over between the two (figs. 28, 29, 31, 36, and 42), which will account for my argument that plastosomes should be deemed the matrix of the lipid formation. And, for the second reason, I will give the fact that the plastosomes, either being considerably reduced in their quantity or having gone out of existence, as the lipid formation progresses, are scarcely detected. Such is the fact which is often noticed in all the cell groups other than the stroma cells of villi, and a part or the whole of the plastosomes cannot but be seen as having been consumed

or exhausted in the formation of lipoids. However, in the Langhans' cells, the stroma cells of villi, and in the decidual cells sometimes, when the functions have advanced considerably, the plastosomes not only show no sign of their decrease, but also increase and present more or less conspicuous groups in a limited section of the cell body. This apparently contradicts the statement given above, but, practically, the reverse is the case. It is probable that the plastosomes consumed partly by the functions performed, are increased and replenished, providing for the repetition of secondary and tertiary functions; by such an assumption the significance of the increase of plastosomes in these cases will be made naturally clear, so that the various images described above do support with more force, instead of contradicting, the theory mentioned above.

And then, the lipid granules growing and enlarging, as they do, from the very small granular bodies described above, change more or less in quality at the same time, and their color becomes somewhat faint, and, moreover, in certain cells, as for instance in a part of the epithelium of the uterine gland and the large-type decidual cells, they sometimes appear as granules having a very clear yellowish-brown color. At any rate, when they reach a certain degree of development, these lipid granules create more or less conspicuous halos around themselves, which gives them the appearance of the contents of vacuoles. Such appearances are very commonly noticed in all the cell groups I have examined, and I cannot help recalling to mind the observations made by Babkin, Rubaschkin, and Ssawitsch respecting pancreatic cells as cited before. Therefore, I believe that this appearance has a very great significance in the secretion formation, in the same way as the phenomena of secretion of the pancreas as interpreted by these three observers just referred to does. In other words, the lipoids may be compared with the secretory granules of ordinary cells, and they like the latter are slowly liquefied, in accordance with the third one of the various forms of glandular secretion (liquefaction) described above, and pass over to the secretions of a vacuolar shape. Therefore, the vacuoles are, after all, nothing else but a modified

product of lipoids, and the contents should possibly be secretions as transparent as water.

According to the arguments given above, the various cell-groups of the placenta and decidua entirely agree with the observations of the glandular cells not only in their structure, but also in the histological changes that follow their functions, and, therefore, there is no room for doubt that secretion should be existent in them also. And it will be briefly stated, concerning their secreting phenomena, that probably lipoid granules rise directly from plastosomes, and then the former, growing in size, slowly change to the vacuoles, viz., secretions, and are thus thrown out of the cell body at times. If looked at from the standpoint of their secretion formation these cells, for the most part, closely resemble the external secretory cells, but viewed with regard to their inner structure in which they keep secretions within their own bodies for a comparatively long period and thus for the most part present a more or less conspicuous foamy image, they should be rather compared with the various internal secretory cells, which are observed in the ovary and the cortex of suprarenals. The principles of the phenomena of secretion, as aforesaid, look very simple indeed, and yet these phenomena do not make their appearance in one and the same cell necessarily at the same time. On the contrary, it is customary that within different parts of the same cell body the various stages of phenomena appear, one after the other, in consequence of which the structure of each individual cell becomes comparatively complex and diverse. Each individual cell, while repeatedly performing its secreting functions in this manner, gradually increase in its size, and it is customary for the cell to grow considerably as it reaches the height of secretion. Even the syncytium layer whose cell border is indistinct, is generally very thick at the height of secretion, and the gradual increase in the size of the cell along with the rise of its secreting functions in this manner may be partly due to the accumulated assemblage of the secretions, though at the same time it cannot be denied that the rise of secreting functions is attended by the increase of the protoplasm and the growth of the nucleus.

There is, of course, a certain limit to the growth of each cell, but there is something exceptional about the decidual cell. It rises from certain extraordinarily small spherical cells within the proper mucous membrane of uterus, and yet it grows so very rapidly and becomes enormous in size that the classification between the large and small types in the ordinary decidual cells, if dealt with according to their genesis, should be anything but significant. For these two mutually run over to one another through the intervention of the intermediate type, and no sharp demarcation exists between them. Therefore, these two kinds, histogenetically, belong to exactly the same kind of cell, and they only differ in that one is still young in its growth while the other has already perfected its growth. However, it must be noted here particularly that the two present an entirely different appearance histologically, and, therefore, in all probability, along lines of their physiological functions. In other words, the decidual cell entirely changes its structure and functions along with the perfection of its growth. That is to say, the decidual cell which has perfected its growth no more demonstrates within its body any important tissue ingredients, except plastosomes; however, it seems that probably, at this period, the cell prepares, by means of the special action of a very strongly developed cell membrane, certain secretions, and sends them forth into the interstitium. At any rate, the cell passing through this stage gradually withers and becomes smaller.

The secretions, while at the height of their formation, are conglutinated with one another, produce in abundance vacuoles of various sizes and shapes, and will show a high beehive structure. And the way of their removal and absorption, if in the syncytium layer, will be, undoubtedly, by rupture, sooner or later, toward the intervillous spaces, and thus they will be absorbed in the mother's blood, while in the various other cells, there is no knowing but that the secretions are thrown out by osmosis, and the secretions of the Langhans' cells and the stroma cells of villi should, as a matter of course, be absorbed on the side of embryo, with the exception of those, which, finding their outlets in the Langhans' islets, are probably taken in by the mother's

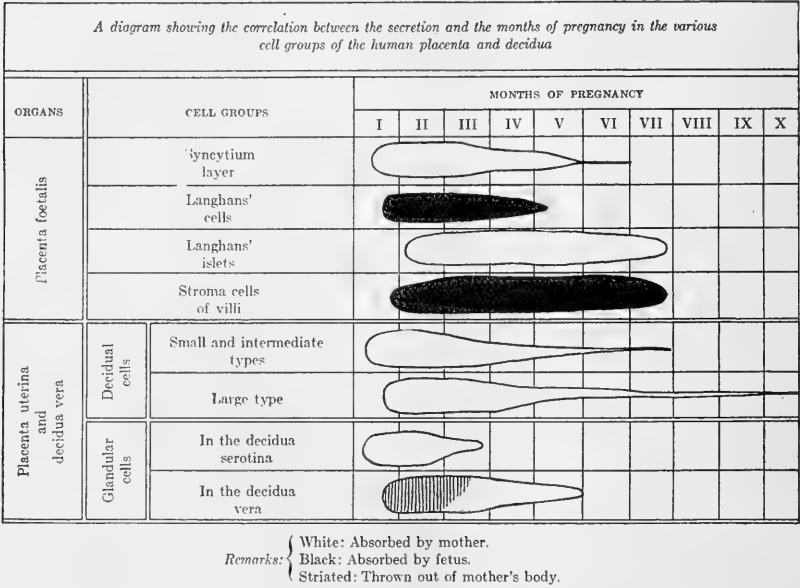
body. Both the large and small types of decidual cells certainly belong to the mother's side, and the secreted or broken-up matter of the uterine glandular cells is at first probably drained into the uterine cavity, to be absorbed by the mother's side. And, on comparing the relations between the secretion of these various cells, and the time of pregnancy we find that, in general, the secretion is at its height in the first half of pregnancy, and especially in the early part of that period, whereas in the second half of pregnancy it generally declines considerably, it being possible to demonstrate it only for a certain period in the stroma cells of villi, the cells of Langhans' islets, and in the decidual cells. Below I will give this correlation with a diagram.

In short, it may be deduced that all the important tissue elements of the placenta and decidua, if looked at from the histological view-point, perform secreting functions. Pending further investigations in all possible directions, it would be difficult to tell what significance these secretions thrown out of the various cell groups have physiologically; however, since it is evident that almost all of their secretions are internally rejected and are taken in either by the mother's or by the fetal side, it makes one feel that, either by the coöperation of certain 'hormones' which should of necessity be contained in each kind of secretions or by their contending actions, both the mother and the fetus would enjoy an extremely delicate and special physiological action. If that is so, it follows that the placenta should contain a great variety of 'hormones,' and the kind and quantity of 'hormones' contained should naturally differ according to the period of pregnancy and the kind of tissues, it being quite clear from the following chart that, speaking generally, those that are found in the early part of pregnancy should be comparatively numerous in kind and in abundance. On the contrary, however, I could not find any important secretions in the placenta which is well ripened. This is the point to which I should like to call attention for the deliberate consideration of all observers who are interested in the placental poison.

THE MINUTE HISTOLOGICAL STRUCTURE AND PHENOMENA OF  
INTERNAL SECRETION IN THE UTERINE MUCOUS  
MEMBRANE PRIOR TO MENSES

1. *My own observations*

a. *The changes of the interstitial cells (figs. 84 to 91).* The one shown in figure 84 is an interstitial cell in normal condition, it is very small and ball-shaped, and the cell body as compared with its nucleus exceedingly small, containing within a certain quantity of plastosomes which are largely rod-shaped.



The nucleus is extremely clear and contains a nuclear network and conspicuous nucleoli. Figure 85 is remarkably larger than the former and is oval. The protoplasm increases in quantity and so does the nucleus. Within the cell body there are no plastosomes to be found, but, on the contrary, there are, in large numbers, strongly black-stained and almost equally shaped lipid granules. The cell illustrated by figure 86 is filled by large numbers of vacuoles within the cell body, and very little is protoplasm proper. The plastosomes, being rod-shaped, for

the most part lie scattered in the partition walls between the vacuoles. Besides, there are, in large numbers, strongly black-stained and almost equally shaped lipid granules. The cell illustrated by figure 86 is filled with large numbers of vacuoles within the cell body, and there is little protoplasm proper. The plastosomes, being rod-shaped, for the most part lie scattered in the partition walls between the vacuoles. Besides, there are, in various parts of the cell body, a few extremely small lipid granules, small in numbers. The nucleus is somewhat dark and the nuclear network is indistinct. In this cell and those that are enumerated below there is a somewhat distinct border membrane on the surface.

In figure 87 both the cell body and nucleus are oval and, though the structure of the cell body is similar in general to the former, the lipid granules appear in a somewhat larger quantity, in some cases existing as the contents of a vacuole. Now, the vacuoles grow larger than in the former in general. The plastosomes are comparatively few. In figures 88 and 89 both the cell body and nucleus are somewhat dark in color. Within there are vacuoles which appear in comparatively small numbers. The plastosomes in the one are somewhat larger in quantity and are distributed all over, while in the other they are comparatively smaller in number and are confined to a certain section. Both demonstrate more or less lipid granules of various sizes. In figure 89 some of the lipid granules contained are light-colored, and it is extremely remarkable to find the manner in which they present themselves as contents of vacuoles. Figure 90 illustrates changes of a very high degree, and the cell body is filled up with remarkably large numbers of vacuoles of different sizes, while the plastosomes lie scattered, in somewhat large numbers, in the partition walls of the vacuoles. The lipid granules are extremely few in number and are very small in size, while the nucleus is grown in size considerably, is clear and has nuclear network and nucleole, both of which are distinct. Figure 91 also shows nearly the same structure as the former, and yet its vacuoles being agglutinated with one another in large numbers, form large and irregular-shaped

cavities, in consequence of which the cell body appears as though it were on the verge of destruction. There are no plastosomes to be detected, though the nucleus appears in a still full and stained condition, and both the nuclear network and nucleoli are conspicuous.

*b. The changes of the glandular cells (figs. 92 to 96).* Figure 92 illustrates, for the sake of comparison, a normal glandular cell, the nucleus is remarkably long and occupies the middle part of the cell body, so that the cell body is divided into the upper and basal parts, each being filled up with numberless plastosomes. The cilia are somewhat short and thick and are not altogether normal. Figures 93 to 96 illustrate the changes which take place prior to menses. Figure 93, as compared with normal conditions, is remarkably larger and its nucleus, being relatively small, lies rather inclined to the base of the cell, while the plastosomes, being chiefly short and rod-shaped, largely lie scattered between the nucleus and the top of the cell, it being a peculiarity of this cell that there are large numbers of yellowish-brown lipid granules assembled at its basal part. Besides, there are in another part of the cell a few deep-back lipid granules, and, again, in this cell there are extremely large numbers of vacuoles nearly of an equal size, crowding together close to the top, viz., the ciliary layer of the cell body, though some vacuoles arrange themselves along the surface of the nucleus in the deeper part of the cell. In the cell illustrated by figure 94, the upper part of the cell is clear, in general, because of the particularly conspicuous vacuolar formations, whereas the common protoplasm is accumulated more or less in the basal two-thirds of the cell, viz., around the nucleus, in which part vacuoles are also detected, though they are for the most part very small. Besides, in this protoplasmic part there are extremely large numbers of plastosomes, which arrange themselves and crowd together in various directions. Again, yellowish-brown lipid granules are found in comparatively small numbers in the basal part of the cell, while deep-black lipid granules, small in size and numbers, lie scattered in the upper part of the cell. The



nucleus is relatively clear, and its nuclear network is indistinct. It is easy to find the traces of cilia in figures 93 and 94. In figure 95 there are absolutely no cilia to be found, and the upper third of the cell is remarkably clear and is formed by somewhat large numbers of vacuoles, whose partition walls, having disappeared in part, give them the form of very irregularly shaped inner spaces. The lower two-thirds of the cell consist of remarkably dark protoplasm, and has in the middle a somewhat large nucleus. Within the protoplasm there are numberless vacuoles of a small size and comparatively small numbers of plastosomes. Both the nuclear network and nucleoli are very conspicuous. And this kind of cell is to be noticed in greatest numbers during the changes of the glandular epithelium. The cell shown in figure 96 is very weak in staining properties, both in its cell body and nucleus, and its minute structure is by no means ascertained. This kind of cell is very seldom seen, and may probably belong to the regressive type.

## *2. The phenomena of internal secretion*

The so-called menstrual decidual cells are extremely varied in their shape and size, and yet, if looked at from the minute histological structure of the cell body, it will be noted that plastosomes, lipoid granules, and vacuoles constitute their chief components. The manner in which the latter, probably following the functions of the cell, correlate with one another may be easily recognized as being in extreme agreement with what is in the small-type decidual cell during pregnancy, and consequently, there is no room for doubt that the functions of the cells concerned are performed in the same manner as the latter. Therefore, not only am I inclined positively to assert the existence of internally secreting functions even in the menstrual decidual cells, but also I believe that the origin of these cells is found in the interstitial cells proper of the uterine mucous membrane, from which origin, gradually with the rise of the function of secretion, a remarkable development and increase of the nucleus and cell body such as is described above are

brought about, the relation in this case being in exact coincidence with the growth of the pregnant decidual cells. These facts taken into consideration, I am convinced that the two kinds of decidual cells (menstrual and pregnant) described above have the same origin, and yet the cells being influenced by the physiological conditions sometimes develop into the menstrual decidual cells, and sometimes, being advanced further, run over to the pregnant decidual cells.

And, on taking a glance at the epithelial changes of the uterine gland, we find that, as in the ordinary glandular cells, plastosomes, lipoid granules, and a large number of vacuoles, which last may be deemed a modified product of lipoid granules, are contained therein. The vacuoles grow in size gradually and are finally fused and present a honeycomb structure, especially on the surface of the cell, and then after losing the cilia, the cells assume the appearance of goblet cells which have their secreted matter accumulated chiefly on the surface. Along with such changes, it will be noted, on the other hand, that plastosomes and lipoid granules gradually diminish and disappear, and it appears that part of those cells which show changes in a high degree die and perish. In short, these structural changes cannot but clearly indicate the fact that these cells perform functions which are similar to the ordinary glandular cells. And, on comparing these changes with those experienced in the glandular cells during pregnancy, we find that the backwardness in the degree of the appearance of lipoid granules and vacuoles occurring in these cells makes one feel as though a decided difference would exist between the two, however true it may be that no radically great difference exists between them.

With regard to the periodic changes of the uterine mucous membrane, there have been many researches, such as the investigations of Hitschman, Adler, and Schröder ('07). Though a universally well-known fact, and yet confined chiefly to the shape of the glandular tubules and the epithelium, very few observers have so far paid attention to the functional significance of the so-called menstrual decidual cells which are produced by the evolution of the interstitial cells, with the ex-

ception of Asada, who has quite recently demonstrated the existence of fat within the cells concerned, and inferred only that this fat is not a degeneration product and must have some relation to the functions of the mucous membrane. However, according to my observations mentioned above, it is easy to clearly recognize that, according to their structure, these cells also have secreting functions as in the case of the ordinary decidual cells. And, looked at from the histological view-point, I do not hesitate conclusively to pronounce that this function declines and terminates immediately upon the beginning of the menses. From want of suitable materials on hand, I am not able to make a definite statement as to what destiny should befall these cells; however, I quite agree with the observations of other investigators in that they suddenly diminish and perish with the arrival of the menses. And, since it is doubtless true that the secretions of these cells are absorbed in the mother's body, it should be a matter of special interest to consider the several clinical symptoms which present themselves frequently at menstruation, in the light of this fact for the explanation of their causative relations. On the contrary, the changes of the uterine glandular epithelium, if compared at the time of pregnancy are remarkably small, and as we can easily assert that its secretions are thrown out of the body, there is certainly no need for argument that it is impracticable to attach an internal secretory significance to the glandular cells; therefore, I am inclined to believe that this sort of periodic changes of glandular epithelium should be recognized as a mere preliminary behavior which is antecedent to pregnancy, and that by far the greater significance, rather theoretically than functionally, should be attached to it.

#### CONCLUSION

1. The epithelium and stroma cells of villi, decidual cells, and uterine glandular cells, all of which constitute the chief tissue elements of the placenta and decidua, if subjected to the closest cytological investigations, show within the cell bodies, and common to them all, the formative constituents, such as

plastosomes, lipid granules, and vacuoles. These constituents, along with the functions of the cells, mutually show the requisite correlation with which they are connected with one another. Specifically:

a. The plastosomes, though for the most part rod-shaped, are either long or short, but occasionally they are granular, chain-like, or filar in their shapes. Their quantity generally more or less diminishes along with the progress of the secreting functions.

b. The lipid granules are extremely varied in their shape, quantity, and in color (in the stained preparations), according to cell or the group to which the cell belongs or perhaps in accordance with the difference in the period of functions. In the earliest stage of their appearance they are always granular-shaped of extremely small size, and sometimes it is difficult to distinguish them from the granular-shaped plastosomes ('plasto-chondrin'), insomuch so that it suggests that the plastosomes may exist in a direct formative participation as matrix of the lipid granules. And this connection is most conspicuously demonstrated in the Langhans' cells, the stroma cells of villi, and in the decidual cells, and even in other cells it is quite easy to recognize it, because the plastosomes tend to diminish more or less in inverse proportion to the increase in the quantity of the lipoids.

c. The vacuoles are probably nothing but the lipoids gradually liquefied and increased into what they are. And, with the rise of functions, they keep increasing in numbers and, as a higher degree of activity is attained, the vacuoles grow in size, and part of them by degrees become agglutinated with one another, so that at last the cell body presents in its entirety a highly foamy image, being composed of numberless vacuoles of various sizes.

The various cell groups mentioned above, if looked at from their minute structure as well as the changes in the formative components, which latter probably have an intimate connection with their functions, bear a close resemblance to the ordinary classical glandular cells (pancreas, salivary and lacrimal

glands) and the important internal secretory cells (luteal and interstitial cells of ovary, the cortical cells of suprarenals), and there exists no radically great difference between the two. That is to say, suppose we now take lipoid granules for secretory granules and vacuoles for secretions, and naturally these cell groups in placenta and decidua should come under the same category as glandular cells, and there would be no doubt whatever that the former have certain secreting functions in themselves.

2. The secreting phenomena of placental and decidual cells, with only the exception of the large-type decidual cells, generally present themselves as in the case of the ordinary glandular cells, with the changes which commonly appear in the structure of the cell bodies and almost under the same form. Now, looked at from the histological viewpoint, the secretions probably rise from the 'plastochondrin,' and then first passing through the period of minor granules which corresponds to Heidenhain's 'Primargranulis,' they gradually grow in size and form into the ordinary lipoid granules, which latter, being liquefied continuously, change directly to the secretions (vacuoles). And, in this matter, it seems that the series of histological changes ordinarily even in the same cell body take place at different times and in different regions, so that the changes make their appearance in repetition secondarily, thirdly, and so on, which fact is responsible for the intricacy of structure which sometimes occurs in certain cells.

3. The methods of discharging secretions, if in the syncytium layer, are that the vacuoles finally rupturing themselves in several parts of the superficial layer cause their contents—secretions—to escape directly into the intervillous spaces in a striking manner, though in the other cell groups the secretions for the most part cannot but be recognized as passing out by 'osmose.' And, of all the secretions, it should be noted that those which come from the syncytium layer, decidual cells, uterine glandular cells (a part) and also probably from the Langhans' islets are absorbed by the mother's body, while those which pass from the ordinary Langhans' cells and the stroma cells of villi are absorbed in the fetal side.

4. As regards the relation between the secreting functions and the time of pregnancy:

*a.* The secreting functions of the syncytium layer may be demonstrated from the beginning of pregnancy to about the end of the fourth month, and yet it is in the second and third months that they are most active.

*b.* The secreting functions of the Langhans' cells are almost entirely the same as in the syncytium layer. It is in the Langhans' islets alone that they last somewhat longer, it being possible to demonstrate cells which have secretions in them up to the fifth or seventh month, and naturally it can be imagined that the functions continue up to that time.

*c.* The secreting functions of the stroma cells of villi begin at about the end of the first month of pregnancy, and keep quite active up to about the seventh month, though from the second to the sixth month they are at their height. However, it should be noted with care that in the eighth month these cells suddenly diminish remarkably and perish, in consequence of which the functions also will drop promptly at this period.

*d.* The decidual cells are entirely different in their appearance falling in the classification into large and small types, as it is well known. That is to say, in the so-called small-type cells the secreting conditions pretty well agree with those in the other cells. This kind of cells appears already quite active on about the seventeenth or eighteenth day after conception, and nearly at the end of the first month of pregnancy its growth and, consequently, its functions reach their climax. Thereafter, as the large-type decidual cells appear, the small-type cells suddenly diminish in quantity, and in consequence it appears that the functions also drop quickly, though even up to the seventh month of pregnancy it is able to clearly demonstrate the existence of the functions.

Then, in the so-called large-type decidual cells, for the most part few, are the structures of the cell body by which the existence of secreting functions may be proved; however, in its strongly developed cell membrane a certain substance is formed, probably by a peculiar faculty of its own, and in this manner

there occurs a material formation which may be deemed a secreted matter which is excreted by the cell body.

The large-type decidual cells are remarkable in their appearance by the end of the first month of pregnancy, though in the second month they appear to reach their climax, and in the following third or fourth months, they diminish in their size. And, the afore-mentioned secreting phenomenon which is peculiar to these cells, begins in the second month, appears most remarkably in the third month, and may be demonstrated up to about the sixth month, though in the seventh month and after it is no longer possible to observe it. In general, the large-type cells retrograde and decay remarkably in the second half of pregnancy, though at the end of pregnancy it is still able to find them, and, moreover, at this period there are some few cells which do contain a small quantity of lipoids.

The functions of the glandular epithelium are most active at the end of the first month of pregnancy, begin to drop considerably from the beginning of the third month, in the fourth month the decline is greater, and in the fifth month, it appears, they almost come to a standstill. In general, the functions make their appearance somewhat earlier in the decidua serotina than in the decidua vera, and accordingly they stop earlier in the former than in the latter. The secretions are thrown out into the uterine cavity probably only in the earliest period of pregnancy, and later as the openings of the glandular tubules are closed by the placental formation and by the adhesion of the decidua vera and decidua reflexa, the secretions, along with the detrital matters of the degenerated glandular cells are, of necessity, absorbed by the mother.

5. Since it is possible that the secretions of the various kinds of cell groups mentioned above are, for the most part, absorbed, either by the mother or by the fetus, as in the case of internal secretions, everybody will easily assent to the supposition that, like the secretions of many internal secretory glands, each of them contains a certain hormone, and should this be the case, it may be said that each of the two organs concerned is assuredly a producer of hormones of various sorts and kinds, and is

also a reservoir for them; and the kinds of hormones and the proportion of their mixture as contents of these organs should have important bearings upon the time of pregnancy and the part of the organs concerned which is taken as material for investigation. And, in the first half of pregnancy, it is possible to show quite a variety of hormones, whereas in a well-ripe placenta it is almost impossible to demonstrate their existence.

6. It has been believed by several authors that the epithelium of villi probably serves as an organ by which nutrition is taken to the embryo; however, histologically it is impossible to find any ground for such argument.

7. The various cells described above usually increase in size more and more as their secreting functions progress. This fact is most remarkably noticed in the decidual cells. The large-type cell is, after all, nothing but the small-type cell grown up; its growth being gradual along with the progress of its functions and with its largest size it has perfected its development. Therefore, though at a glance it would seem that these two kinds of cells are entirely different from one another, yet they have the same origin, and originally they are the cells of the same kind. However, the sharp demarcation which exists between the two functionally should deserve our attention; that is to say, the decidual cell performs the conversion of its functions along with the perfection of its growth.

8. The foregoing conclusions would, at a glance, seem to contradict the work conducted by several authors up to now whose conclusion it was almost entirely to deny the internal secreting functions of the placenta and decidua; but the main reason for this is the fact that the materials employed for investigation by these authors have been for the most part mature organs, for in these there is almost no proof of any secreting phenomena being existent, and they have been therefore taken at most unfavorable times as materials to help us attain our aim. Therefore, in the future, should anyone desire to try his hand in this sort of research, it would be necessary for him by all means to take materials while yet in the early



part of pregnancy. And, even in this case secretions of several kinds, even if they come from cells of one and the same origin, would possibly be, by no means, similar in quality, but rather in the organ concerned, there would be existent various kinds of substances produced from the various cell groups which form the organ. And, in case that there is a certain hormone action in these substances, it would follow that, during a certain period of pregnancy, the hormones will act upon both the mother and the fetus in diverse and complex manners.

9. The histological changes which the interstitial cells of the uterine mucous membrane and glandular cells undergo prior to menses resemble, in general, the changes which take place at the beginning of pregnancy, though they are by far the weaker. Therefore, even in that case, these two kinds of cells, looked at from their histological structure, have in common to themselves, secreting functions, to whose existence we may positively assert. And, the secretions, if in the interstitial cells, are undoubtedly absorbed internally, as in the case of the small-type decidual cells while in pregnancy, and should thereby bring about the various clinical symptoms which are experienced during menses.

The glandular cells differ from the former, and the secretions have probably no endocrine nature and are immediately thrown out to the outside, viz., into the uterine cavity, so that it would be difficult to attach to them an important physiological significance, such as hormone action. Rather, it would be fit to interpret such periodical changes of these cells as preliminary phenomenon of the coming pregnancy.

10. The interstitial cells of the uterus, prior to menses, are developed into the so-called menstrual decidual cells, which in point of structure, distinctly reminds us of the decidual cells of pregnancy. For this reason, it would be in order for us to trace the origin of the latter, as of the former, to the interstitial cells of the uterus.

In conclusion, my whole-hearted gratitude goes to Prof. R. Tsukaguchi for the kind and sincere leadership and revision which he has given me at the present work, and to Professor Ogata, chief of our gynecological department, for the valuable materials for research and for the immense assistance he has given me.

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#### EXPLANATION OF PLATES

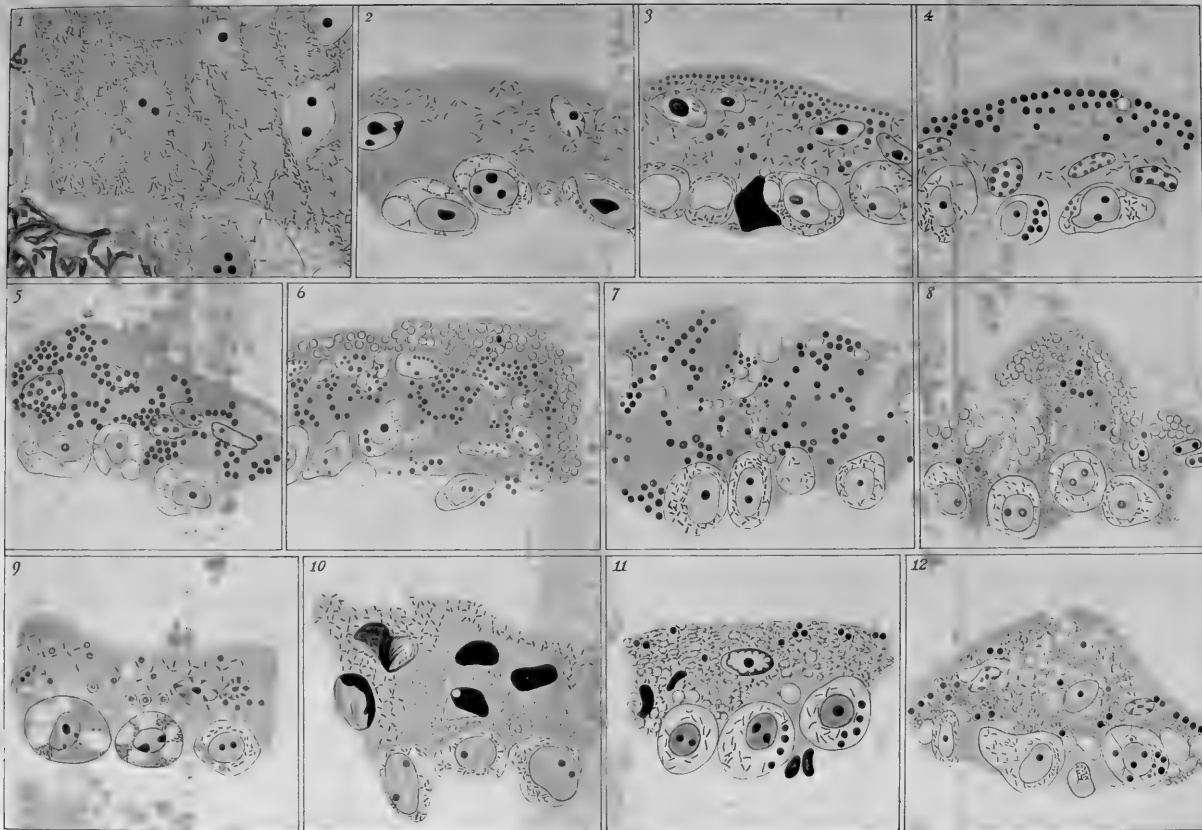
All the figures given on plates have been drawn at the height of the object stage, by Abbe's apparatus, under the same magnifying power: Zeiss' apochromat homogeneous immersion 3 mm., compensations okular. 12, tube length 160 mm.

The various figures have all been drawn from the preparations fixed by Levi's solution and stained by Heidenhain's iron-alum-haematoxylin, with only the exception of figure 7, which has been reproduced from the preparations by Altmann's method, after changing the color.

PLATE 1

EXPLANATION OF FIGURES

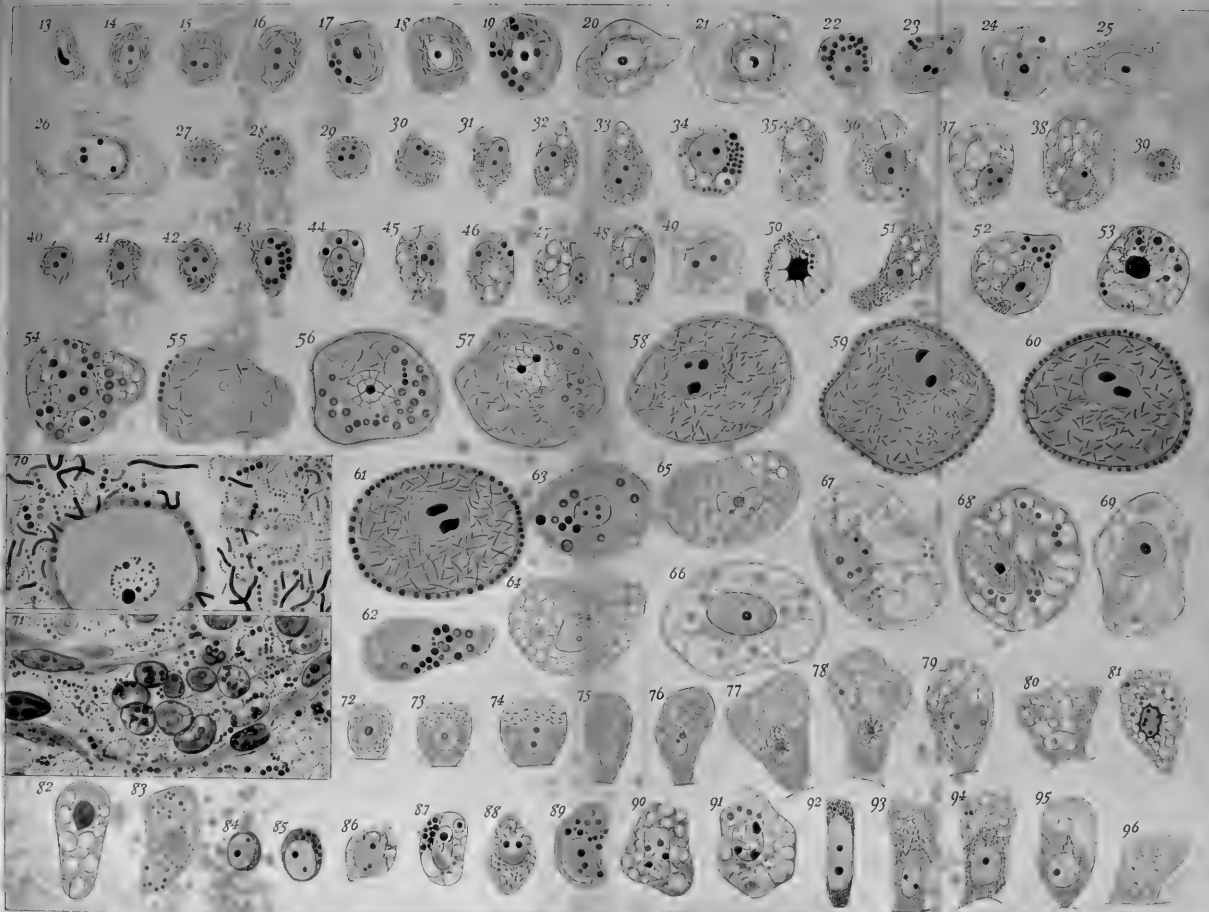
1 to 12 Illustrate the syncytium layer and a part of the Langhans' cells.



## PLATE 2

### EXPLANATION OF FIGURES

- 13 to 26 Illustrate the Langhans' cells.
- 27 to 38 The stroma cells in the chorion villi.
- 39 to 69 The decidual cells.
- 70 The peculiar-looking product which makes its appearance in the cell membrane and interstitium of the large-type decidual cells.
- 71 The above-mentioned product filling up the blood vessels.
- 72 to 83 The glandular epithelium during pregnancy.
- 84 to 91 The interstitial cells prior to menses.
- 92 to 96 The glandular epithelial cells prior to menses



Resumen por el autor, Albert M. Reese.

La estructura y desarrollo de las glándulas tegumentarias de los Crocodilia.

El presente trabajo versa principalmente sobre *Alligator mississippiensis*, suplementado por algunas observaciones llevadas a cabo sobre el caimán, *Caiman* sp. de la Guyana Inglesa. El autor discute el desarrollo y la estructura adulta de tres series de glándulas, a saber: Las pequeñas glándulas dorsales que constituyen dos filas debajo de ciertas escamas dorsales desde la mitad de la región cervical, próximamente, hasta la región de la cloaca; las glándulas mandibulares, las cuales poseen un orificio bastante grande situado ventralmente en cada lado de la mandíbula; y las glándulas cloacales, un par de grandes cuerpos ovales que se abren en la cloaca. Las glándulas mandibulares y cloacales sirven para la secreción de una substancia azmizcosa y probablemente son más activas durante la época de la cría. La función de las numerosas glándulas dorsales, más pequeñas, es problemática.

Translation by José F. Nonidez  
Cornell Medical College, New York



## THE STRUCTURE AND DEVELOPMENT OF THE INTEGUMENTAL GLANDS OF THE CROCODILIA

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SIX PLATES (FORTY-THREE FIGURES)

### MATERIAL

The greater part of the material upon which the embryological portion of this paper was done was collected by the author some years ago in Georgia and Florida, under a research grant from the Smithsonian Institution; several other papers have already been published with this material as the foundation. The caiman material here used was collected by the author in British Guiana during the summer of 1919, under a grant from the Carnegie Institution of Washington; the species of caiman that laid the eggs could not be determined.

The embryos were fixed in various ways (in sublimate-acetic more than in any other fluid), were stained mostly with borax carmine and Lyons blue, and were cut transversely, sagittally, and frontally.

The structures to be described are the dorsal glands and the musk glands.

### THE DORSAL GLANDS

The dorsal glands of the alligator extend from the cloacal region to about the midcervical region. As seen in figure 1, they lie beneath the anteromesial corner of the second row of scales from the middorsal line. When a piece of the skin of a young animal, preserved in fluid, is viewed by transmitted light, the glands appear in the form of circular areas, as seen in figure 1, which represents two rows of dorsal scales from the back of a 200-mm. alligator, in the region of the pelvic legs.

The structure of the adult glands will be taken up after a discussion of their development.

The gland was first seen in an embryo of about 25-mm. crown-rump length (fig. 2) as a rounded thickening and invagination of the lower layer, stratum germinativum (fig. 3 and fig. 4, *s*). As may be seen in figure 3, the gland lies somewhat above the level of the dorsal side of the spinal cord, *sc*, and is relatively of large size. It extends nearly through the dermis or corium, *c*, to the outer edge of the underlying muscle, *m*.

Under moderately high magnification the epidermis, at this stage, is seen to consist of two layers, a very thin, outer layer or periderm (Krause) (fig. 4, *p*) and a much thicker, inner layer or stratum germinativum, *s*. Beneath the latter lies the very thick and fairly compact and homogeneous layer, the corium, *c*. The outer layer, *p*, exaggerated in distinctness in figure 4, is very thin and contains flattened nuclei. The inner layer, *s*, is of somewhat variable thickness and consists of one or two layers of polyhedral or cuboidal cells; the nuclei of these cells are oval or spherical and frequently lie at the base of the cells.

The gland, *g*, at this stage consists, as has been said, of a thickened invagination of the stratum germinativum. This thickening consists (fig. 4, *g*) of a mass of indistinctly outlined polyhedral cells, with large, spherical or oval nuclei. At the fundus or inner end of the gland the cells are more closely compacted than in the region nearer the surface. A wide invagination, *d*, of both layers of the epidermis represents the position of the future wide-mouthed duct of the embryonic gland.

The next stage represented in figure 5, is taken from an embryo of a total length of a little over 7 cm. The epidermis and corium are here about as in the preceding stage, but the gland, *g*, is much more elongated and now projects into the underlying muscular tissue, *m*, as a bottle-shaped mass of cells, connected with the superficial epidermis by a rather narrow neck. The open invagination of the epidermis, *d*, is here deeper and narrower than in the preceding stage.

The cells of the gland have about the same appearance as before, but they are distinctly less compactly arranged in the

center of the gland than nearer the periphery, thus foreshadowing the condition seen in the mature gland. The cells are small, compared to the size of their nuclei, and of indistinct outlines. In pushing down into the underlying muscular tissue the gland has carried with it a thin surrounding layer of the corium as a sort of connective-tissue sheath or capsule, *cp*.

The next embryo studied (fig. 6) had a total length of about 13 cm. The gland here (fig. 7) is distinctly flask-shaped, the neck of the flask forming the wide duct, *d*, which is plugged with a fairly compact mass of cells. The more bulging side of the fundus is toward the median plane of the animal, to the right in figure 7. The fundus of the gland, *g*, projects well down into the underlying muscular tissue, *m*, and is surrounded by a thick, though loose, connective-tissue capsule, *cp*.

The epidermis, *ep*, contains numerous pigment granules that mask somewhat the cell details. It extends down into the duct to about the region where the enlargement to form the gland proper begins; here it gradually becomes thinner and finally merges into the cell mass of the gland. As seen in figures 7 and 8 there are irregular spaces among the cells of both gland proper and duct. The cell details at this stage are very difficult to determine with accuracy.

Figure 8 represents a section through the fundus of the gland, six sections anterior to the one shown in figure 7. The gland, which is now beginning to approach the adult structure, consists of a mass of irregular, often elongated, cells, with oval nuclei. Around the periphery is a more or less distinct row of nuclei, belonging to a sort of layer of basilar cells, to be noted in a later stage. In the irregular central cavity or lumen may usually be seen a few scattered cells; whether these are artificially torn off or are regularly desquamated from the underlying cells it is not easy to determine. The lumen, *lu*, if it may be so called, being so irregular in outline, it is not possible to say just how many layers of cells make up the wall of the gland. In some places the lumen extends to within one or two cells of the periphery; in other regions there are six or more irregular layers of cells. Further discussion of these cells will be

deferred to later stages; they are evidently not yet functioning, and would not be expected to function this long before hatching.

The dorsal gland of a 15-cm. embryo is shown in figure 9, as drawn with a camera under medium magnification. The epidermis, *ep*, here exhibits two well-defined areas; the above-mentioned periderm, which now shows clearly, under high powers, as a fibrillar or scaly structure, and the stratum germinativum, *s*, from which the gland has been developed, as described above. In the stratum germinativum, as as well as in the immediately subjacent corium, *c*, may be seen numerous brownish pigment bodies of irregular shape and size.

In the region of the duct, *d*, which lies in the longitudinal groove between two scales, the corium is considerably thickened. In the particular section here shown the opening of the gland to the surface, is not shown and in fact, this gland did not show any opening at all, the periderm being uninterrupted. In other series a break in the periderm was visible and an irregular opening through the thickened area of the stratum germinativum could be made out. There is, however, quite a sharp line of demarkation between the closely packed mass of cells of the stratum germinativum, that forms the apparent duct of the gland, and the more loosely arranged, less deeply staining cells of the gland proper. While the gland proper is now several times larger than it was at the preceding stage, the duct is relatively and actually considerably narrower and shorter.

The gland, as may be seen in figures 1 and 9, is circular in outline and is flattened until its dorsoventral thickness is about one-half its diameter. It lies almost entirely below the corium, *c*, and is surrounded by a thin connective-tissue capsule, *cp*. It consists of a fairly compact peripheral mass of cells, which become looser and more scattered towards the center (fig. 9). These cells are finely granular and have such indistinct walls that their boundaries can only with great difficulty be determined; they contain oval or round nuclei. Around the periphery of the gland, next to what may be called the basement membrane, is a fairly distinct row of nuclei, representing an indistinct layer of basilar cells, (figs. 9 and 10, *b*); passing from these cells towards

the center or lumen of the gland, *lu*, the nuclei become more and more scattered and the cell walls less distinct, until the cells are quite indistinguishable and appear as an irregular, granular mass with an occasional nucleus, *nu*.

Figure 11 represents a portion of the wall of the dorsal gland of a 1-meter alligator, as seen under a magnification of 270 diameters. The glands, which are here about 3 mm. in diameter, were dissected from the skin, and hence do not show the duct. The structure is quite similar to that of the stink gland of the turtle, *Terrapene odorata*, as figured by Dahlgren and Kepner ('08).

The capsule, *cp*, consists of a loose layer of elastic fibers, *ct*, with numerous nuclei, surrounded by a muscular layer, *m*, of varying thickness, which is made up of two more or less distinct layers of involuntary fibers, running in different directions.

In the gland proper is seen a great mass of irregular and spherical cells, while the intercellular spaces and lumen (if it may be so called) are filled with the granular secretion.

The peripheral cells, just beneath the capsule, form a narrow but usually fairly distinct layer, the basilar cells, *b*, mentioned in the preceding stage. The basilar cells are small and clear, their large oval or spherical nuclei filling a large part of the cells. It is probably by the division of these cells that the other cells, with their contained secretion, are produced. There is never more than one layer of basilar cells, and even in this layer the cells are rather scattered, though always close to the capsule.

Next to the basilar cells is a generally fairly distinct layer of much larger cells, *gs*, *gs'*, presumably those last formed by division of the basal cells. In some regions this second layer consists of swollen spherical cells, *gs*, with granular contents and peripherally located nuclei; in other places these cells are distinctly columnar or cuboidal in shape, *gs'*, due, possibly, merely to crowding. In these cells may occasionally be seen a spherical clear area, probably an oil droplet, *od*.

The main body of the gland is made of a scattered mass of cells, secretion, degenerate nuclei, etc. Some of these cells, *o*, seem

to be filled with a fat or oil; they are clear and spherical, with a much flattened nucleus just beneath the cell membrane. A majority of the cells, *o'*, while spherical in shape, still contain a greater or less amount of granular protoplasm, in which the nucleus lies. While an occasional cell, *gs''*, may be seen in which the entire content is granular; most of these are indistinct in outline and of moderate size. A number of irregular cells, with little or no contents may be seen, *cs*, which have the appearance of being empty cell membranes; these may be the remains of cells from which the granular or the fatty secretion has been emptied.

Numerous nuclei, *nu*, may be seen scattered among the cells; since they are not shrunken, but are usually well formed, they may be those that have been extruded in the breaking down of the granular rather than of the oil cells.

What the function of these dorsal glands may be it is difficult to surmise. No odor was detected in connection with them such as evident with the submandibular and cloacal musk glands. Their small size and wide distribution over the dorsum of the animal might indicate that they are of use in keeping the scales in good condition, making them comparable to the oil glands in the skin of mammals.

Possibly in the living animal the dorsal glands may have an odor and may function as accessory musk glands, though, as as stated above, no odor has ever been noticed by the present writer.

#### THE MUSK GLANDS

According to Gadow ('01):

All the recent crocodilia possess two pairs of skin-glands, both secreting musk. One pair is situated on the throat, on the inner side of the right and left half of the lower jaw. The opening of the gland, visible from below . . . , is slit-like, and leads into a pocket, which in large specimens is the size of a walnut; the bag is filled with a smeary pale brownish substance, a concentrated essence of musk, much prized by natives. The secretion is most active during the rutting time, when the glands are partly everted. My young Crocodiles and Alligators often turned them inside-out, like the finger of a glove, when they were taken up and held by force. The other pair lies within the lips of the cloacal slit, and is not visible from the outside. The use of these strongly scented organs,

which are possessed by both sexes, is obviously hedonic. The sexes are probably able to follow and find each other, thanks to the streak of scented water left behind each individual.

#### CLOACAL MUSK GLANDS

As in the skunk and other animals, the alligator possesses a pair of well-developed glands, one on each side of the cloaca, into which they open. These glands, like the dorsal glands, are developed from the lower layer of the epidermis, and are first seen in embryos of slightly more than 7 cm. total length (fig. 12). In these embryos the penis (fig. 12, *pe*) is a large, thick organ projecting markedly from the cloaca to the exterior. As seen in figures 12 and 13, the glands are thickenings and slight invaginations, *cg*, of the epidermis in the lateral walls of the cloaca, *cl*, rather nearer the surface than the bottom of the cloaca; they are at some distance posterior to the opening of the rectum into cloaca. The epidermis, *ep*, is somewhat thicker in the cloaca than over the general body surface, consisting in the former region of two or three layers of cells instead of the single layer, beneath the periderm, seen over the body. The gland now consists of the above mentioned thickening of the lower layer of epidermis (fig. 13, *cg*) which is slightly invaginated and consists of about six or eight layers of cells, somewhat more loosely arranged near the surface than around the periphery or deeper part of the thickening. The periderm, *p*, in some sections seemed interrupted at the point of invagination, but it is seen to extend to the very bottom of the duct in later stages, this interruption was probably an artifact or an optical illusion.

Opposite this point of origin of the gland the penis, *pe*, showed a slight thickening or bulge of its wall.

Figure 14 shows the cloacal region at a slightly later stage of development. The gland, *cg*, is somewhat more elongated in shape and exhibits a lumen of considerable depth, slightly bifurcated at its bottom. The relation of parts will be understood by comparing this figure with figure 12. This embryo was about 9 cm. long, from tip of snout to end of tail, measured along the greater curvature of the body. The next stage is an embryo of

about 15 cm. total length. The cloacal glands (fig. 15, *cg*) are considerably more developed than in the preceding stage; the gland on the right side is here seen cut through its median region; the other through the opening of the duct, *d*, into the cloaca, *cl*. The body of the gland is elliptical in shape, being somewhat greater along an anteroposterior diameter than along the lateral or the dorsoventral diameters. A slight condensation of the connective tissue around each gland indicates the beginning of the capsules, *cp*.

The gland is still a solid mass of cells, though the cells in the central region are somewhat larger and clearer than those around the periphery. The duct, as seen on the left side, is open for only a very short distance.

Figure 16 represents a section through the cloacal region of a slightly later stage of development than the last.

The cloacal gland, *cg*, is here cut through its duct *d*, which is rather long and narrow. The epidermis, *ep*, is thrown into complicated folds and is of fairly even thickness throughout; it is directly continuous with the peripheral cells of the gland proper. The periderm, *p*, is seen along the free border of the epidermis throughout its many folds. It may be traced along the inside of the duct into the gland, where it is lost. Beneath the epidermis, in the outer part of the corium, are seen numerous brownish pigment granules, *pg*, that are especially numerous around the periphery of the gland proper, in what may be considered the capsule. Outside of the connective tissue part of the capsule is an irregular layer of muscle fibers, *m*. In the center of the gland is an irregular cavity to be noted in connection with the high-power drawing. A section through the line *a-b* in figure 16, drawn under a magnification of about 600 diameters, is shown in figure 17. The layer of pigment granules surrounding the gland is shown to the right, *pg*; the letter *a* is placed in the edge of the central cavity of the gland.

Three regions of cells may be made out, though they are not always sharply differentiated from each other. The outer layer, next to the pigment granules, is composed of rather small, irregular cells, with smaller nuclei than those of the other layers.



Inside of this layer is the middle layer, composed of distinctly larger cells, elongated in form and with distinct, heavy walls. The nuclei of these cells often do not stain, so that the layer has a clear appearance due to the absence of visible nuclei. The inner layer, bordering the central cavity, is the thickest of the three in most places. It is composed of large, irregularly spherical or polyhedral cells, fairly sharply differentiated from the middle layer. The cytoplasm is more scant and scattered than in the other layers, and the nuclei are very variable in size, some of them being large and spherical, others being less than half as large and of a shrunken appearance. Next to the central cavity these cells are quite irregular, with jagged edges as though they were being torn off from the rest of the cell mass.

Figure 18, A, represents a lateral view of a cloacal gland of an 80-cm. caiman, freed of the surrounding muscles and loose connective tissue. It is retort-shaped with a maximum length of 1 cm. and a greatest width of 6 mm. The neck of the retort is, of course, the duct of the gland. The gland actually shown in figure 18, A, was cut sagittally and is shown in figures 19, 20, and 21. The connective tissue covering the capsule over the gland proper is compact and rather thin, but, as will be seen in the description of the sections, around the duct it is a very thick, rather loose mass, so that the diameter of the duct is not nearly so great as it would appear in this surface view of the gland.

Seen in sagittal section (fig. 19) or in transverse section (figs. 22, 23 and 24), the gland is found to contain a large lumen, perhaps filled with secretion, which is eccentrically located and from which the duct opens to the surface of the cloaca.

Figure 19 represents a longitudinal section through the median plane of the gland shown in surface view in figure 18, A.

The duct, *d*, is cut almost exactly through its median plane and shows on the right the complicated folds of its walls where it opens to the surface. These folds or wrinkles resemble the more complicated wrinkles seen in the duct of the mandibular gland, to be described later, which seems to turn itself inside out. The wall of the duct is composed of eight or ten compact layers of cells, flattening out toward the lumen, where they form a thick layer

of horny, scale-like cells like those of the superficial epithelium with which they are, of course, continuous. The wall of the duct becomes continuous with the cell mass of the gland.

The gland proper (fig. 19, *cg*) as was seen to be the case in the preceding stage, is composed of a compact mass of cells, which under the low magnification used in drawing figures 19, 22, 23, and 24, exhibit a distinct radiate appearance, strands, *st*, of differently stained cells radiating from the lumen of the gland toward the periphery. The character of these cells will be described later.

The capsule, *cp*, consists of a mass of connective-tissue and muscle fibers which is rather thin and fairly compact over the gland proper, but is greatly thickened (as noted above) and looser in character in the region of the duct. Owing to the position of the duct, the surrounding connective tissue in this region is much thicker on one side than on the other, as shown in figures 19 and 22. This connective tissue consists largely of white fibers, with numerous nuclei and small blood vessels. The muscle fibers form a thin layer near the outer gland cells.

At the base of the duct, to the left in figure 19, is a rounded mass of fine granules, *l*, surrounding a small blood vessel; this has the appearance of a lymph node.

Transverse sections through the duct and fundus of the cloacal gland of a 32-inch caiman are shown in figures 22, 23, and 24. Figure 22 passed through the lower part of the duct, *d*, just before it enters the gland proper, *cg*. The duct, which exhibits the thick wall described above in connection with figure 19, contains a mass of lightly stained secretion. Surrounding the duct is the loose mass of connective tissue, *ct*, which is condensed around the periphery to form a more distinct zone than was shown in figure 19 around the entire mass.

Figure 23 passed through the upper part of the gland where the lumen, *lu*, is large and partially filled with secretion. The gland proper shows the radiating strands of more lightly stained cells, mentioned in connection with figure 19, which are wide near the lumen and taper sharply toward the periphery of the gland. The capsule, *cp*, is very well marked.

Figure 24 is through the lower part of the gland proper, the only sign of the lumen being a tiny hole, *lu*, in the center of the gland mass. The strands of cells, *st*, are seen as about twenty circular or oval areas of varying sizes scattered through the glandular area. One of these strands surrounds the remains of lumen, *lu*.

The capsule, *cp*, sends in strands or irregular septa toward the center of the gland. These strands increase in number as the bottom of the gland is approached.

The character of the cells of the gland at this stage, as seen under moderately high magnification, is shown in figures 20 and 21. The regions of the gland from which these cells were drawn are shown by the two circles, 1 and 2, in figure 19.

In region 1, figure 20, which is just beneath the capsule, *cp*, most of the cells seen are of moderate size, very irregular in outline (though tending toward the hexagonal form from mutual compression) with moderate-sized, deeply stained nuclei. These cells are separated into groups of irregular sizes and shapes by fine septa of connective tissue which extend in from the capsule. In the edge of this region is shown the distal, pointed end of one of the radiating groups of cells, *st* (fig. 19), mentioned above. The cells in this group, while varying greatly in size, are on the average much larger than those just described, in some cases being several times as large. The nuclei are proportionately larger also, and the cell walls are very heavy and sharply defined. The cells at the distal end of the group (toward the capsule) are not very sharply differentiated from surrounding smaller cells, as though they were being developed from these cells.

In region 2, figure 21, the cells of the radiating strand, *st*, are sharply differentiated on both sides from the smaller surrounding cells, and both they and the surrounding cells have the same characteristics as noted above for region 1.

At this stage, in those sections that passed through a mass of secretion in the lumen of the gland, a sharp line of demarkation is evident between the cells of the gland and those of the secretion, which is different from the condition to be noted in the next stage.

Figure 18, B, represents a lateral view of the cloacal gland of a 1.6-meter caiman, removed from the body and freed from the surrounding mass of muscle and connective tissue. It is, like the preceding 80-cm. stage, distinctly retort shaped, the duct, *d*, representing, of course, the neck of the retort. The region of the gland from the duct to the dotted line has a dark appearance, as though there were black tissue showing through the capsule; the other half of the gland has a pale yellow color. The gland has a distinct musky odor and an oily feel, and when some of the 80 per cent alcohol in which the gland is preserved is allowed to evaporate on a slide, a film of oil droplets of various sizes (fig. 25) is deposited upon the glass.

A small segment, from the capsule inward, was cut from the middle region of this gland and was sectioned and stained. Figure 26 represents a small portion of this segment, shown under low power, chiefly to indicate the positions of three typical regions which are shown in figures 27, 28, and 29 under higher magnification.

Figure 27 shows a section through the capsule, *cp*, and the adjacent cells, region 1. The capsule consists of outer and inner layers of connective-tissue fibers, *ct*, on either side of a fairly sharply defined layer of involuntary muscle fibers, *m*. It is generally stated that the capsule of the cloacal gland has no muscular layer and that the secretion of the gland is expressed by the muscles of the cloacal region. The gland cells in this region, particularly those nearer the capsule, are small and irregularly arranged, with small nuclei and quite indistinct cell walls. No arrangement of cells comparable to the basilar cells, described in connection with the dorsal gland (fig. 11), can be determined. Strands of connective tissue from the capsule penetrate the gland among these cells at intervals, but are not shown in the figures.

In region 2, figure 28, the cells are much larger, as a rule, than in region 1, usually several times as large. The nuclei also are variable, but generally larger, and the cell walls are very sharply defined, almost like those in some plant tissues. The radiating strands, *st*, noted in connection with the preceding stage are not noticeable here.

Figure 29 shows the character of the cells in region 3 of figure 26, which covers more area of the segment than is shown in the two preceding figures. Toward the periphery of the gland, to the right in the figure, the cells are large and sharply defined, with large, centrally located nuclei, and distinct cell walls, like the cells in figure 28. As the cells are traced toward the lumen of the gland, to the left in this figure, their clean-cut outlines become irregular, the walls being shrunken and more or less collapsed; the nuclei are smaller and less numerous, and are generally located near or against the shriveled cell walls. To the extreme left of the figure, which is practically the boundary of the indefinite lumen of the gland, the cell-mass consists of what would ordinarily be called adipose tissue. No, or few, nuclei are present, and all that is seen is an irregular network of empty cell walls, the oily contents having probably been dissolved out by the preparation of the tissue for sectioning. In the middle of this region, slightly indicated in the figure, the cells are often flattened, as though by pressure from within outward. This flattening might possibly be caused by the pressure of the secretion of the gland held within its central lumen.

#### THE MANDIBULAR GLANDS

The position of the mandibular glands is shown in figure 30, which represents the ventral side of the head of a 20-cm. alligator; this is the size of the animal on emerging from the egg. As seen in figure 30, better in figure 31, the opening of the gland at this stage is a longitudinally elongated slit, lying in a shallow groove and bordered by irregularly elongated scales. In larger animals, perhaps in those of this size, though the writer has never seen it, the alligator may turn the gland partially inside out, as noted above by Gadow, causing it to project above the surface of the scales as a round or oval sort of rosette, with a circular or elongated (fig. 36) central depression. The inside cells thus exposed form an irregular, rough surface, so that the appearance is somewhat like that of a partly expanded sea-anemone. Whether, in their native habitat, the adult alligators evaginate the musk glands whenever the musky odor is emitted, the writer is un-

able to say. The evaginated cells are of a dark, brownish-gray color. When cut from the animal the musk gland, even when the inner cells are evaginated, is seen to project beneath the inner surface of the skin as a smooth, oval mass of considerable size, to be described in connection with the adult animal.

The mandibular musk gland is first seen in embryos of about 25 mm. crown-rump length (fig. 2). A vertical section through the lower jaw of such an embryo is shown in fig. 32. The glands are here seen, *mg*, as wide-mouthed pits formed by the invagination of the ectoderm on either side of the midventral line. The ectoderm increases in thickness from one layer of cells over the general surface to three or four layers at the bottom of the invagination. The pit is here about  $1/10$  mm. wide and  $4/10$  mm. long. A slightly denser area of mesoblast is seen around the bottom of each invagination, possibly the first indication of the capsule of the gland.

Figure 33 represents one of the gland invaginations of an embryo of about the same size, but slightly more developed, drawn under a greater magnification. The invagination is here deeper and proportionately narrower than in the preceding stage. Its bottom or fundus is distinctly enlarged and bulb-like, *mg*, and the rest of the invagination forms what might now be called the duct, *d*. The walls of the duct are of varying thickness and consist of two or three indistinct layers of cells. The fundus has a fairly distinct wall of several layers of elongated cells and a central mass of somewhat scattered and irregularly arranged cells. Surrounding the gland is a distinct area of condensed mesoblast, *cp*. Several small blood vessels are seen, scattered through the mesoblast. Along the dorsal edge of the lower jaw at this stage may be seen numerous somewhat smaller and solid invaginations of ectoderm, quite similar to the one just described; these are the rudiments of the teeth.

Figure 34, from a transverse section through a slightly older embryo, shows but little advance over the preceding stage. The fundus of the gland, *mg*, is shown distinct from the duct, *d*, owing to the plane of the section. The entire gland is larger in size and the surrounding condensation of mesoblast, *cp*, is more

marked, especially in close proximity to the periphery of the gland. The wall of the fundus consists of three or four layers of elongated cells with oval nuclei; the center of the fundus consists of a mass of irregularly grouped cells, with more rounded nuclei and indistinct walls.

In an embryo of considerably larger size, about 15 cm. total length, the mandibular gland seemed practically unchanged except that the duct was longer the diameter of the fundus was slightly greater, and there was a small lumen in the center of the fundus where the irregular mass of cells was seen in the preceding stage.

Figure 35 shows the cell structure of the gland in an embryo at about the time of hatching. The gland has increased greatly in size, though it is probably not yet functioning. The present figure represents a section of the wall of the gland, extending from the surrounding capsule, *cp*, to the central lumen, *lu*.

The capsule now shows both fibrous and muscular layers and will be discussed later. The lumen has the appearance of a small, irregular, torn opening in the center of the gland.

The wall of the gland consists of about a dozen irregular layers of cells, which, for the most part, have thick, distinct walls and large spherical or oval nuclei. Immediately surrounding the lumen, however, there is a granular mass, in the peripheral part of which indistinct nuclei and cell walls may be seen, as though it were composed of broken down cells. Immediately adjacent to the lumen little or no indication of cell structure may be seen. Except for the immaturity of the animal, one might suppose that this central granular mass was the secretion of the gland, formed by the breaking down of the surrounding cells; but as the musk glands are probably of sexual character; they would not be expected to function so long before sexual maturity is reached.

Figure 36 represents a surface view of a partially evaginated mandibular gland of a 1-meter alligator. As noted above, the animal has the power of partially turning these glands inside out, so that they have somewhat the appearance of an expanded sea-anemone. In the specimen shown in figure 36, the rough,

evaginated portion had a deep longitudinal depression in the center. Sections of this gland will be described below.

Figure 37 shows a vertical section through a mandibular gland that had been removed from a 1-meter alligator, freed of all loose surrounding tissue, and cut, free-hand, through the center with a razor. Cell details are not shown, the figure being intended to show the shape of the gland, *mg*, and the enormous mass of the wrinkled and folded evaginated material, *ev*.

Figures 38 and 39 are vertical sections through the mandibular gland of a 1-meter alligator, the former passing through the duct, *d*, of the gland, the latter passing to one side of the duct.

The greatly wrinkled epidermis, *ep*, covered with a loose layer of horny material, *h*, is seen to extend down into the duct, *d*, of the gland where it is continuous with the main cell mass, *mg*. Doubtless, part of the epidermis adjacent to the gland has been pushed to the surface by the partial evagination process. The capsule, *cp*, consists of a loose mass of connective tissue and muscle fibers, the contraction of the latter doubtless causing the evagination of the gland.

The central region of the gland consists of a mass of fibrous tissue (fig 38, *h*, *h'*), continuous with the horny layer of the epidermis. This central mass of tissue is loose and scattered in the center, *h'*, but is compact and dense peripherally, *h*. It is quite sharply differentiated from the adjacent cells of the gland, *mg*.

The main body of the gland consists of a compact mass of cells, *mg*, somewhat like those described in figure 35 and is broken up into irregular lobules, as seen in figures 38 and 39, by strands of deeply pigmented connective tissue from the capsule. The cell details of these lobules will be discussed in connection with the next and last stage of the gland.

Figure 40, A and B, shows the shape and size of the submandibular glands of the adult caiman and alligator, respectively. The former animal, of which the species was not known, was about 1.6 meters in length. The alligator was the American species and was probably somewhat longer than the caiman, possibly 2.5 meters. It will be noticed that while the two glands are



of about the same length, the one from the alligator is more than twice the diameter of the one from the caiman. They have both been freed of the loose, surrounding tissue and are smooth and shiny superficially.

The gland (*B*) from the alligator shows the rosette of evaginated matter, covered with small, usually pigmented, papilla-like projections with an irregular central depression. A section through such a rosette has already been described (fig. 37) for a somewhat smaller animal. The gland proper is white, but has the appearance of a translucent covering over a dark center which shows through faintly.

The gland from the caiman was not evaginated at the time it was killed, so that no rosette effect is seen. The appearance of the dark center showing through the white capsule, noted above, is very marked in the half of the gland next the duct, so that this half of the gland is distinctly darker than the other half. Possibly it is the pigmented mass of unevaginated papillae, seen in *B*, that gives this darker color to the half of the gland next the surface.

Figure 41 is an outline sketch, mostly drawn with the camera lucida, of a vertical section of the submandibular gland of an alligator of a length of somewhat less than 2.5 meters. The section here represented passed near, but not through, the actual opening of the duct to the exterior. The general shape of the gland proper and of its evaginated portion, *ev*, is well shown. In the center of the gland is an irregular lumen, *lu*, partially filled by a mass of material to be discussed below. Extending towards the center of the gland from the capsule, *cp*, may be seen a member of irregular, branching strands of pigmented connective tissue that divide the gland into a number of irregular lobules, *lo*, whose central lumina open into, or are really a part of, the lumen of the gland, mentioned above.

Figure 42 represents the cell structure of a section of the gland between the parallel lines shown in figure 41, *pl*. The inner region only of the capsule, *cp*, is shown on account of the great thickness of this structure as seen under the moderately high magnification used.

The entire thickness of the wall is made up of a compact mass of cells that do not show a very great amount of variation in different regions of the section. The cells adjacent to the capsule are perhaps, on the average, somewhat larger than those nearer the midregion of the section, while the cells adjacent to the lumen of the gland are distinctly larger than the other cells.

The nuclei of the cells vary largely in size, and many of them, particularly in cells nearer the lumen, show distinctly angular outlines, as though they were somewhat shrunk. Many of the cells bordering the lumen are without nuclei, and gradually become more irregular and shrunk as the lumen is approached, until they may become continuous with the irregular mass of secretion in the lumen, *lu*.

The secretion of this gland, as noted above, is a yellowish or brownish, oily mass with a musky odor. Under the microscope (fig 43), this yellowish, paste-like mass is seen to consist of isolated granular cells of various sizes, some with distinct nuclei, some with scarcely visible or no nuclei; surrounding the cells are fat droplets of all sizes, probably derived from the broken-down cells whose shrunk and empty walls may be seen in sections that pass through the lumen of the gland.

#### SUMMARY

Three sets of integumental glands are found in the alligator, the dorsal glands, of uncertain function, and two pairs of musk glands.

The dorsal glands are minute, spherical structures that are found just under the skin in two rows, one row on each side of the middorsal line. They open to the surface through minute pores. They develop as thickenings and invaginations of the lower layer of the epidermis. The wall of the adult gland consists of a sort of loose, stratified epithelium, resembling somewhat the structure of the stink gland of the turtle. Since these glands are very small and have, so far as could be determined, no odor, they probably have some other function.

Of the musk glands, the pair that opens into the cloaca is the larger, though the glands that open by a slit on either side of the ventral wall of the throat are of considerable size in large animals. Like the dorsal glands, the musk glands are developed by an invagination of the lower layer of the epidermis and their walls are composed of somewhat similar though more compact layers of cells. The secretion, or musk, is a smooth, oily substance with the powerful odor characteristic of that well-known extract, and is doubtless used by the sexes, both of which produce it, in locating and following each other during the breed-season when the glands are said to be most active.

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## ABBREVIATIONS

<i>b</i> , basilar cells	<i>m</i> , muscle
<i>bv</i> , blood vessels	<i>mc</i> , Meckel's cartilage
<i>c</i> , corium	<i>mg</i> , mandibular gland
<i>cg</i> , cloacal gland	<i>mg'</i> , opening of mandibular gland
<i>cl</i> , cloaca	<i>na</i> , neural arch
<i>cp</i> , capsule	<i>nc</i> , notochord
<i>ct</i> , connective tissue	<i>nu</i> , nucleus
<i>d</i> , duct	<i>o</i> , fat cell
<i>dg</i> , dorsal gland	<i>o'</i> , young fat cell
<i>ds</i> , dorsal scale	<i>od</i> , oil drop
<i>ep</i> , epidermis	<i>p</i> , periderm
<i>es</i> , empty cell	<i>pe</i> , penis
<i>ev</i> , evaginated part of gland	<i>pg</i> , pigmented granules
<i>g</i> , gland	<i>pl</i> , parallel lines, showing plane of section in figure 41
<i>gs</i> , <i>gs'</i> , <i>gs''</i> , gland cells	<i>r</i> , rib
<i>h</i> , horny layer of epithelium	<i>s</i> , stratum germinativum
<i>h'</i> , central mass of horny layer	<i>sc</i> , spinal cord
<i>l</i> , lymphoid tissue	<i>st</i> , strands of cells
<i>lo</i> , lobule of gland	
<i>lu</i> , lumen of gland	

## PLATE 1

### EXPLANATION OF FIGURES

1 Two transverse rows of scales from the back of a 20-cm. alligator, to show the location of the double row of dorsal glands, *dg*. The top of the figure is cephalad, which shows that the glands are located in the anteromesial corners of the lateral row of scales. Lateral to the four longitudinal rows of large dorsal scales is shown, on each side, the edge of the small scales of the side and belly of the alligator.  $\times 3\frac{1}{2}$ .

2 Lateral view of an alligator embryo of 25 mm. crown-rump measurement.

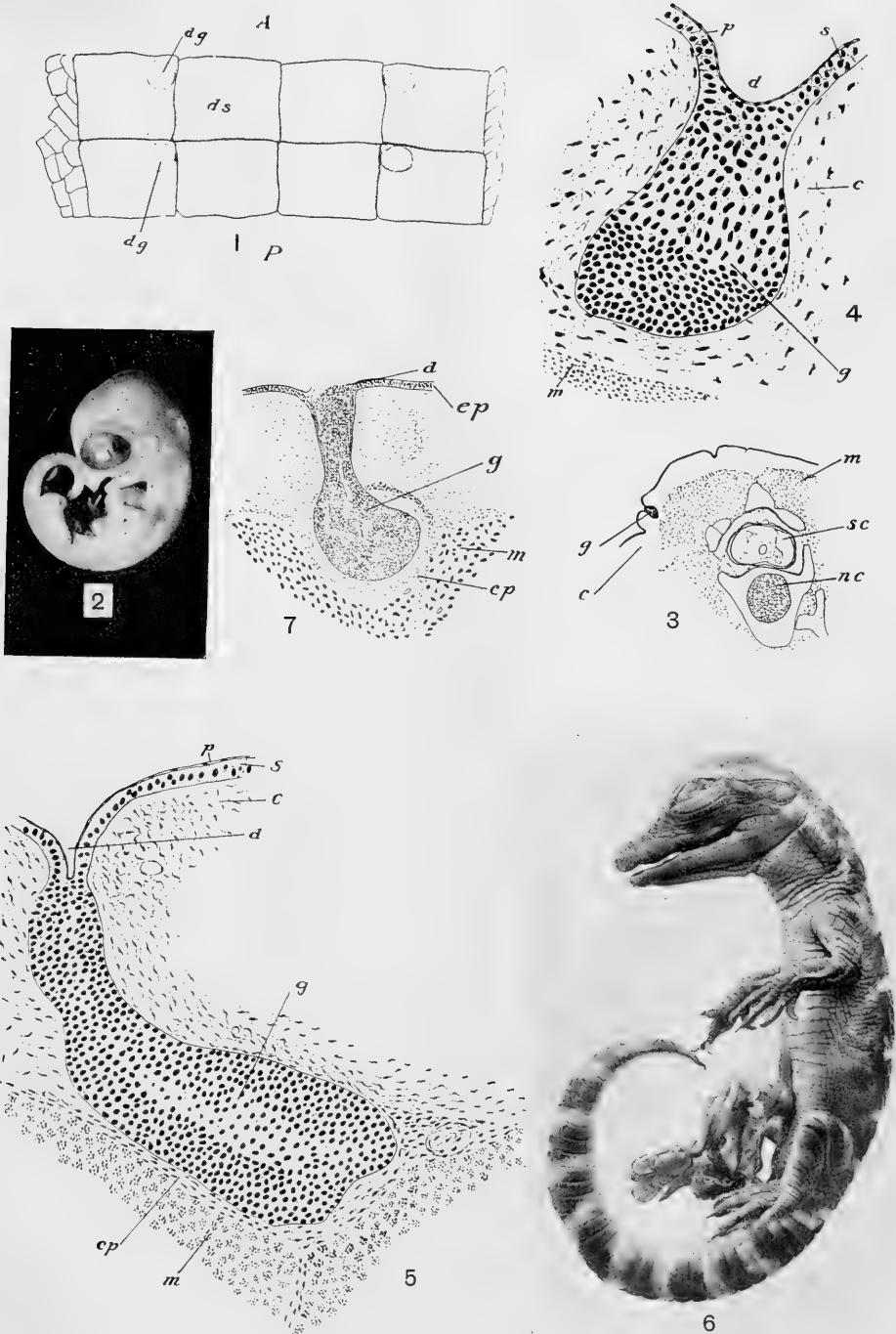
3 Transverse section through the neck region of the embryo shown in figure 2, to show, under low magnification, on the left side, the invagination of the epidermis to form the dorsal gland, *g*.

4 The gland, shown at *g* in figure 3, drawn under higher magnification to show cell structure.

5 Longitudinal section through the gland at a slightly later state of development, drawn under moderately high magnification.

6 Lateral view of an alligator embryo of about 13 cm. total length.

7 Section through the dorsal gland and duct in the pelvic region of an embryo of about 13 cm. total length.



## PLATE 2

### EXPLANATION OF FIGURES

8 Higher magnification of a section through the same gland as shown in the preceding figure.

9 Section through a dorsal gland of a 15-cm. embryo, drawn under medium magnification.

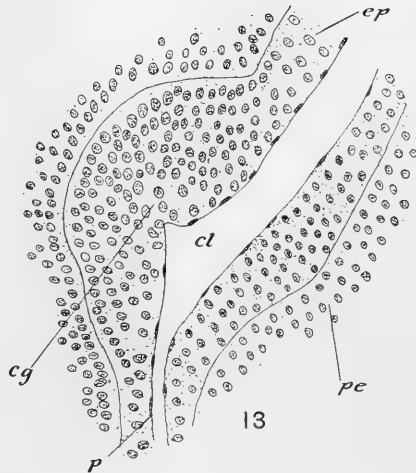
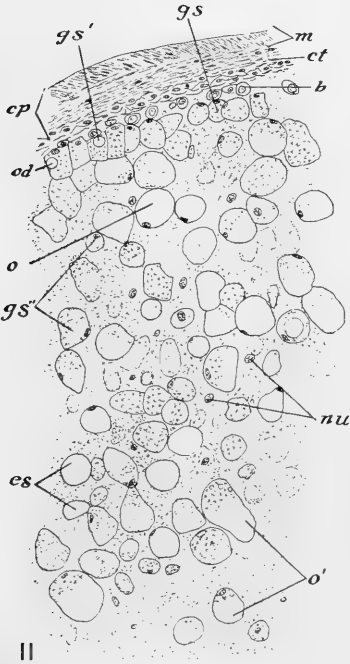
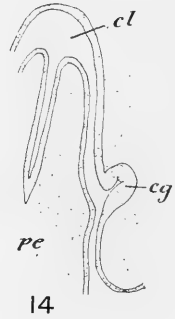
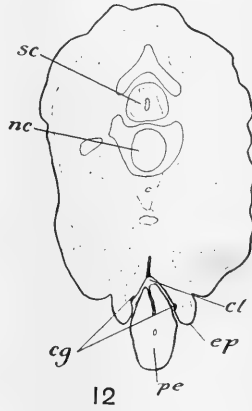
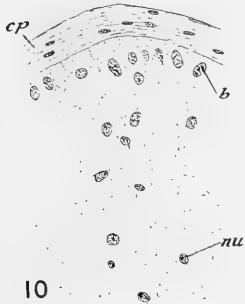
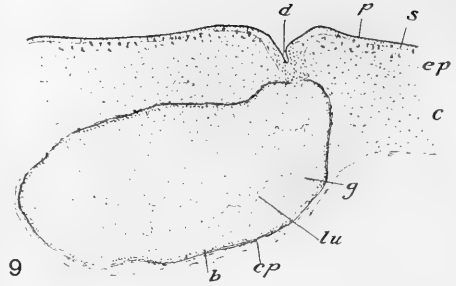
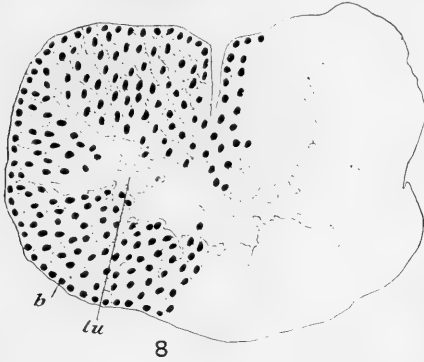
10 Portion of the wall of the gland shown in figure 9, as seen under moderately high magnification.

11 Portion of the wall of a dorsal gland of a 1-meter alligator, as seen under moderately high magnification.

12 Transverse section through the cloacal region and penis of an alligator embryo of about 7 cm. total length. The early invagination of the epithelium of the cloaca to form the cloacal glands is shown, *cg*.

13 A higher magnification of the cloacal gland shown on the left side of the cloaca in the preceding figure.

14 Section through the penis and cloaca of an embryo somewhat more developed than the one represented in figures 12 and 13. The cloacal gland, *cg*, is more deeply invaginated than in the preceding stage.



### PLATE 3

#### EXPLANATION OF FIGURES

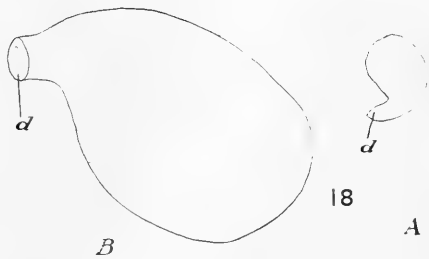
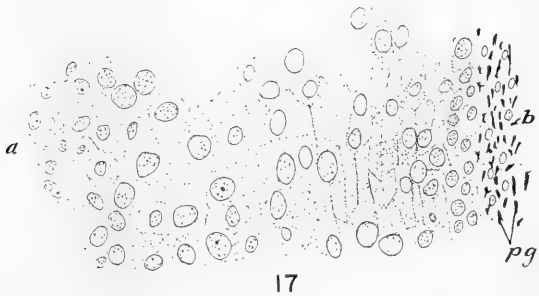
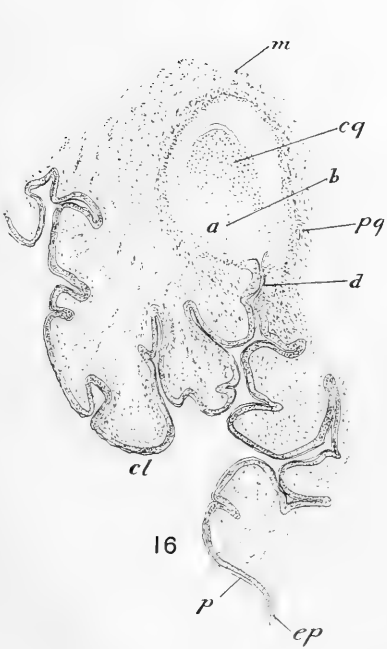
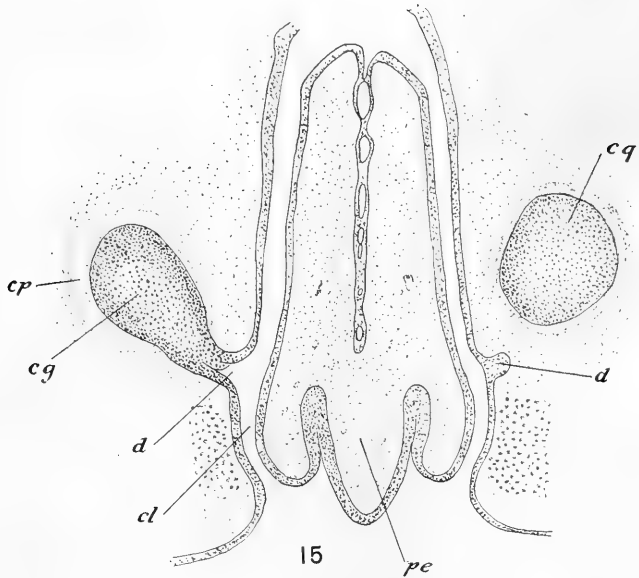
15 Section through the cloaca and penis of an embryo of about 15 cm. total length, from tip of snout to tip of tail. The gland on the left side is cut through its duct, *d*, that on the right to one side of the region where the duct, *d*, connects with the gland, *cg*.

16 Section through one cloacal gland, at a somewhat later stage of development than the last. The distinct duct, *d*, and much-folded epithelium, *ep*, are shown.

17 A section along the line *a-b* in figure 16, drawn under magnification of about 600 diameters, to show the character of the cells of the gland wall.

18 A, cloacal gland of an 80-cm. caiman sp., freed of loose surrounding tissue.  $\times 1\frac{1}{2}$ . B, cloacal gland of 1.6 m. caiman sp.  $\times 1\frac{1}{2}$ .





## PLATE 4

### EXPLANATION OF FIGURES

19 A, median longitudinal section through the gland shown in figure 18, A, as seen under low magnification.

20 High magnification of the gland wall in the region inclosed in circle 1, figure 19.

21 High magnification of the gland wall in the region indicated by circle 2, figure 19.

22 Transverse section through the deeper part of the duct of the cloacal gland of an 80-cm. caiman.

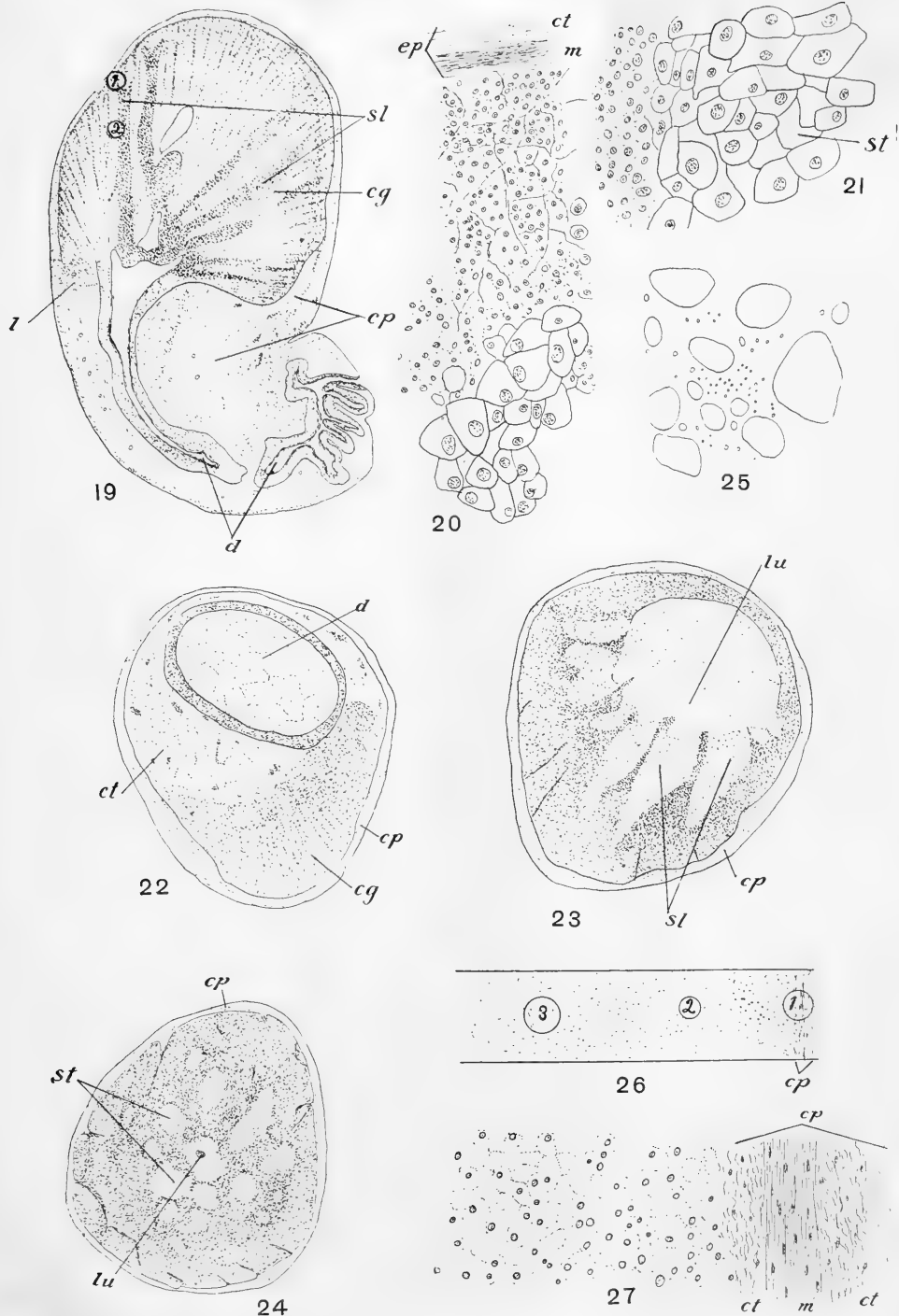
23 Transverse section through the same gland as represented in figure 22, but cutting through the middle region of the gland proper, deeper down than the plane of section 22.

24 Transverse section through the same gland as represented in figures 22 and 23. The plane of this section is still farther from the duct and passes through the deeper region of the gland.

25 High-power sketch of oil droplets extracted with 80 per cent alcohol from the cloacal gland of a 1.6-meter caiman and deposited upon a slide by the evaporation of the alcohol.

26 Low-power sketch of a segment cut from the middle region of the gland shown in figure 18, B. The segment extends from the capsule, *cp*, to the center of the gland, and is represented chiefly to show the three typical regions whose cell details are shown in the following three figures.

27 Details, as seen under moderately high magnification, of cell structure in region 1 of figure 26; this region includes the capsule, *cp*.



## PLATE 5

### EXPLANATION OF FIGURES

28 Details, as seen under slightly less magnification than was used in figure 27, of cell structure in region 2 of figure 26.

29 Details, under the same magnification as used in figure 28, of cell structure in region 3, figure 26.

30 View of the ventral surface of the head of a 20-cm. alligator, showing the two slit-like openings, *mg'*, of the mandibular or throat musk glands.

31 Enlarged drawing of one of the openings shown in figure 30.

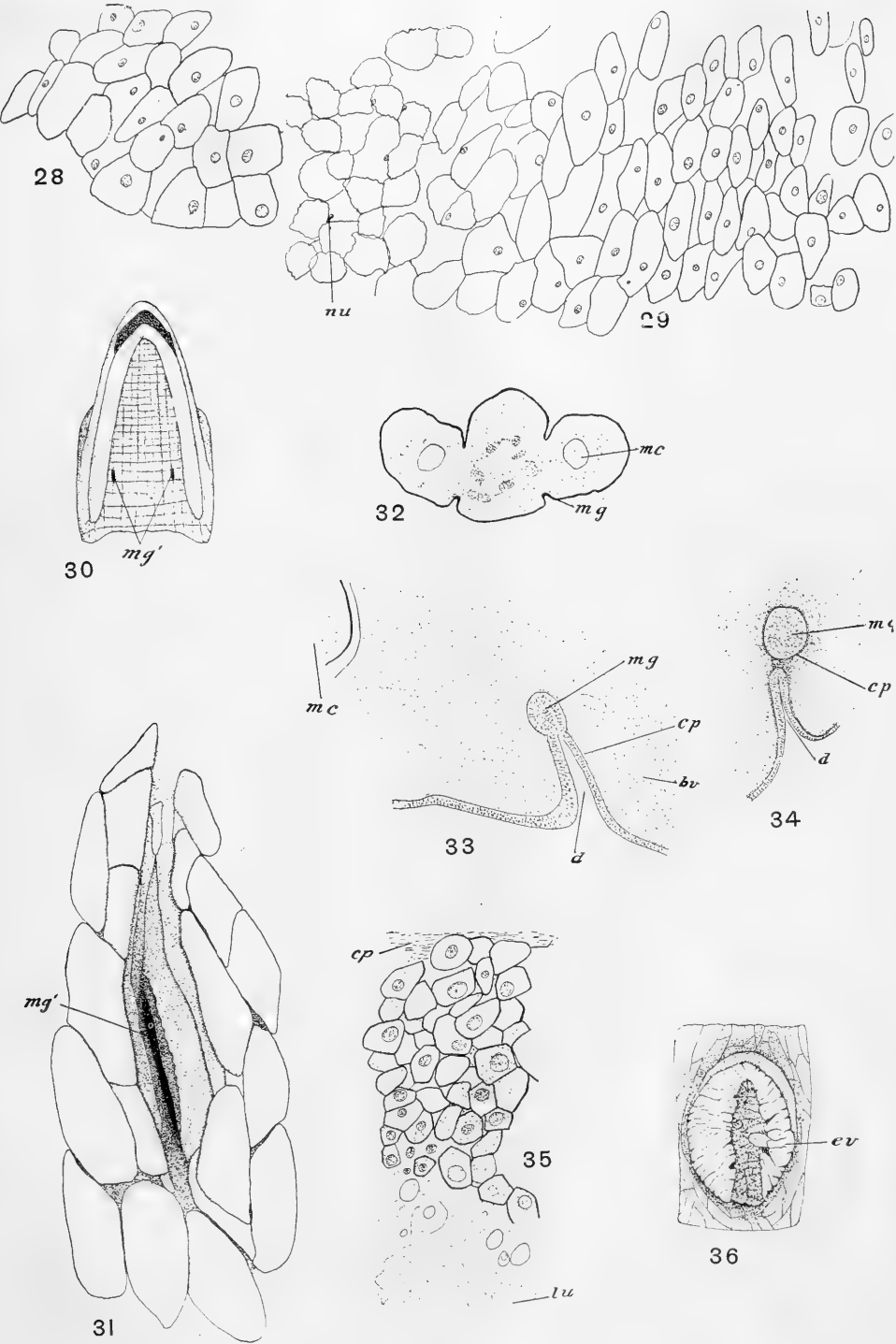
32 Vertical section through the jaw of the embryo shown in figure 2.

33 A somewhat more highly magnified section through the gland invagination of an embryo of about the same size as, but slightly later development than, the one represented in figure 32.

34 Section through the mandibular gland of an embryo of about 4 to 5 cm. length.

35 Section through the wall of the mandibular gland of an alligator at about the time of hatching; under moderately high magnification, to show cell structure.

36 Surface view of a partially evaginated mandibular musk gland of a 1-meter alligator.



## PLATE 6

### EXPLANATION OF FIGURES

37 Sketch of a free-hand section passing vertically through the mandibular gland of a 1-meter alligator. The gland was cut nearly through the median plane. No cell details are shown

38 Vertical section through the mandibular gland and its duct of a 1-meter alligator, as seen under low magnification.

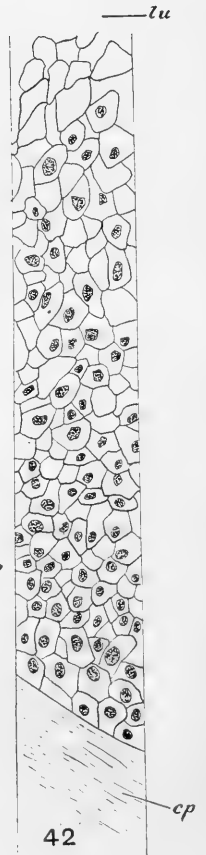
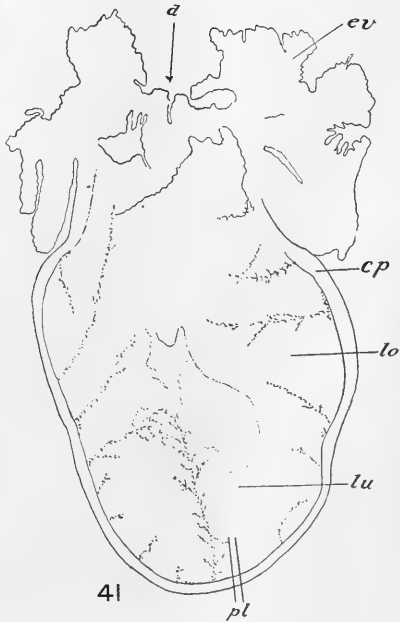
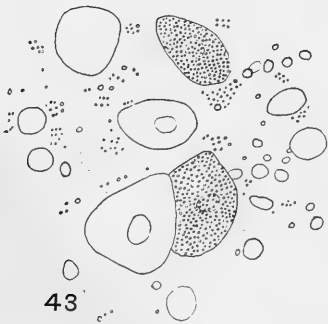
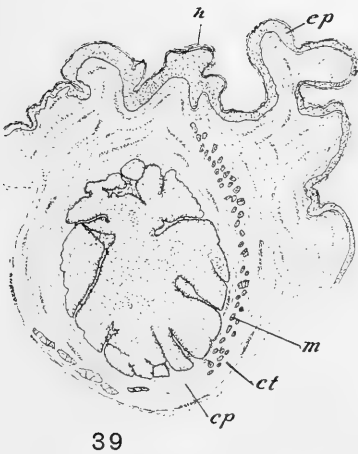
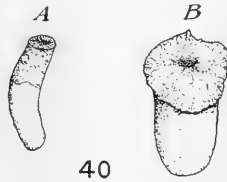
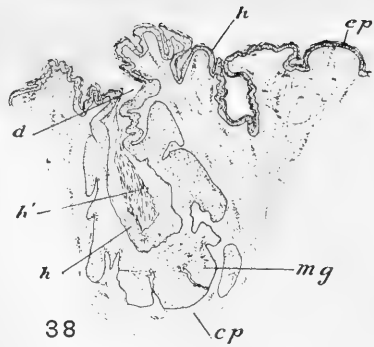
39 Similar section to preceding, but passing to one side of the duct.

40 A, surface view of mandibular gland of caiman *sp.* of about 1.6 meters' length; no evagination of interior seen.  $\times /$ . B, surface view of mandibular gland of an alligator of probably about 2.5 meters' length. Marked evagination of the internal structures shown.  $\times /$ .

41 Outline sketch of a mandibular gland of an alligator of about the size of the one shown in figure 40, B.

42 Cell details of the wall of the gland, between the parallel lines, *pl*, shown in figure 41

43 Highly magnified appearance of some of the yellowish secretion squeezed from the mandibular musk gland of a 2.5 meters alligator.



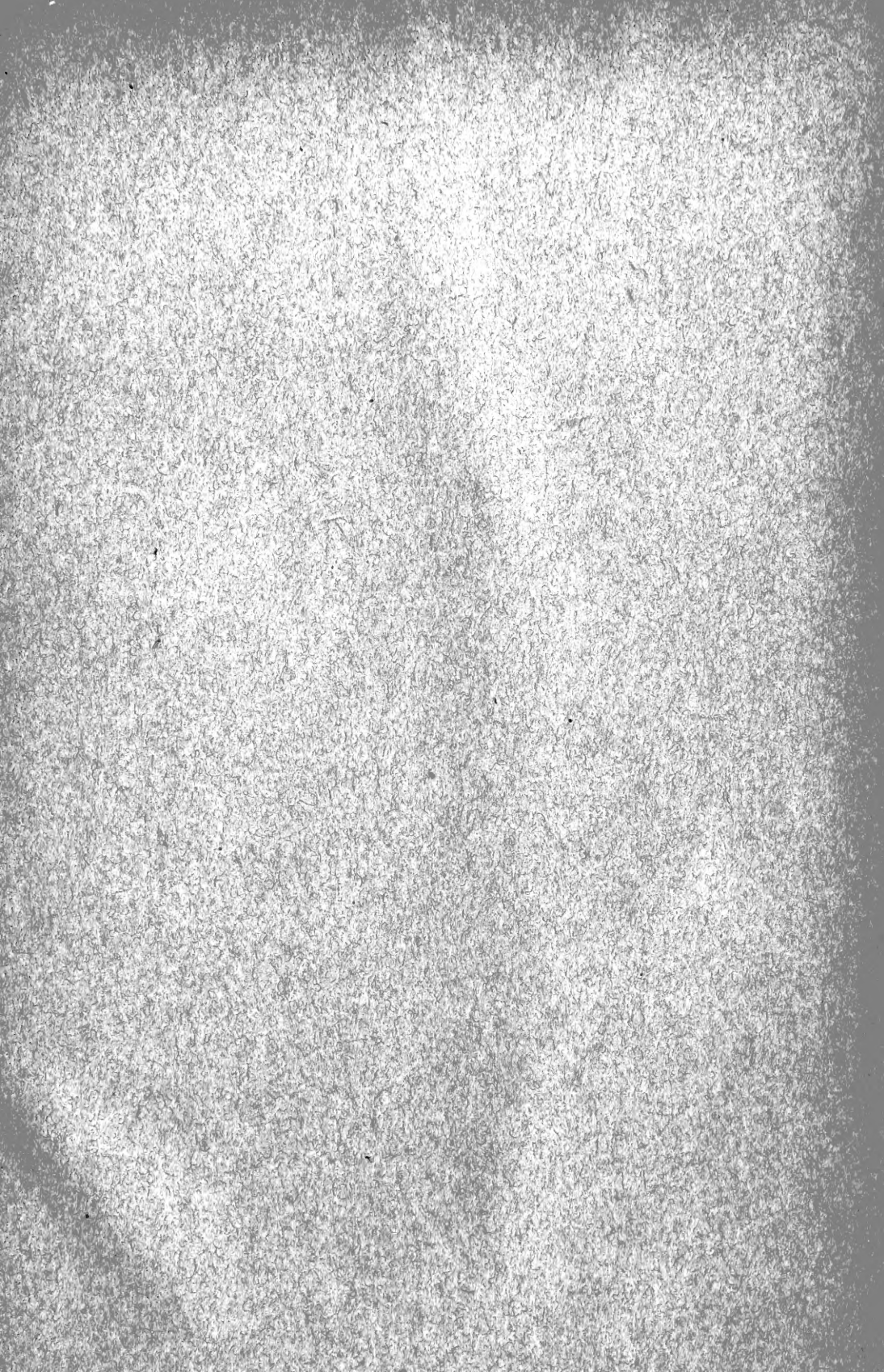


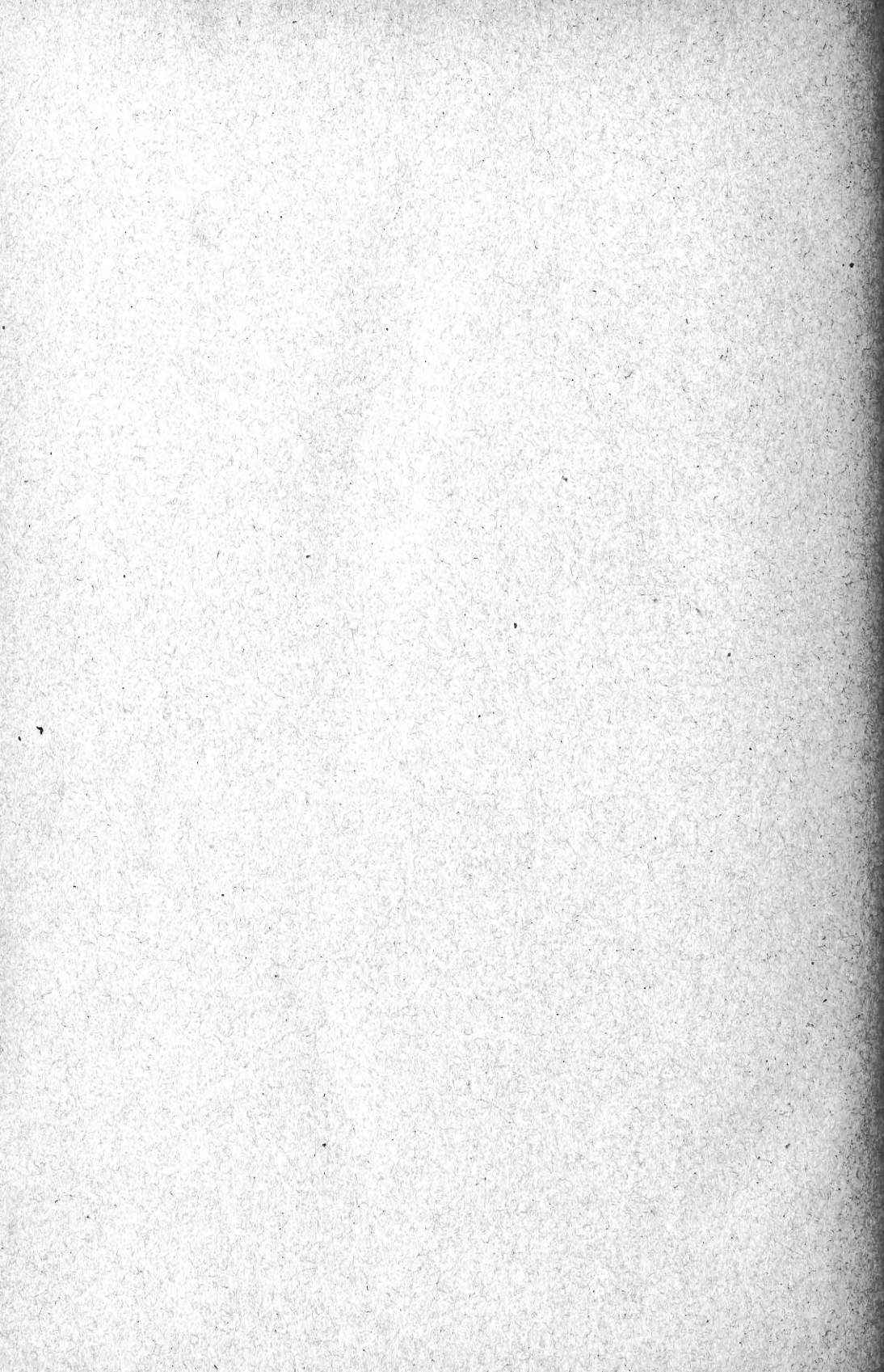


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